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Waterbath stunning of chickens

SIMONE PRINZ

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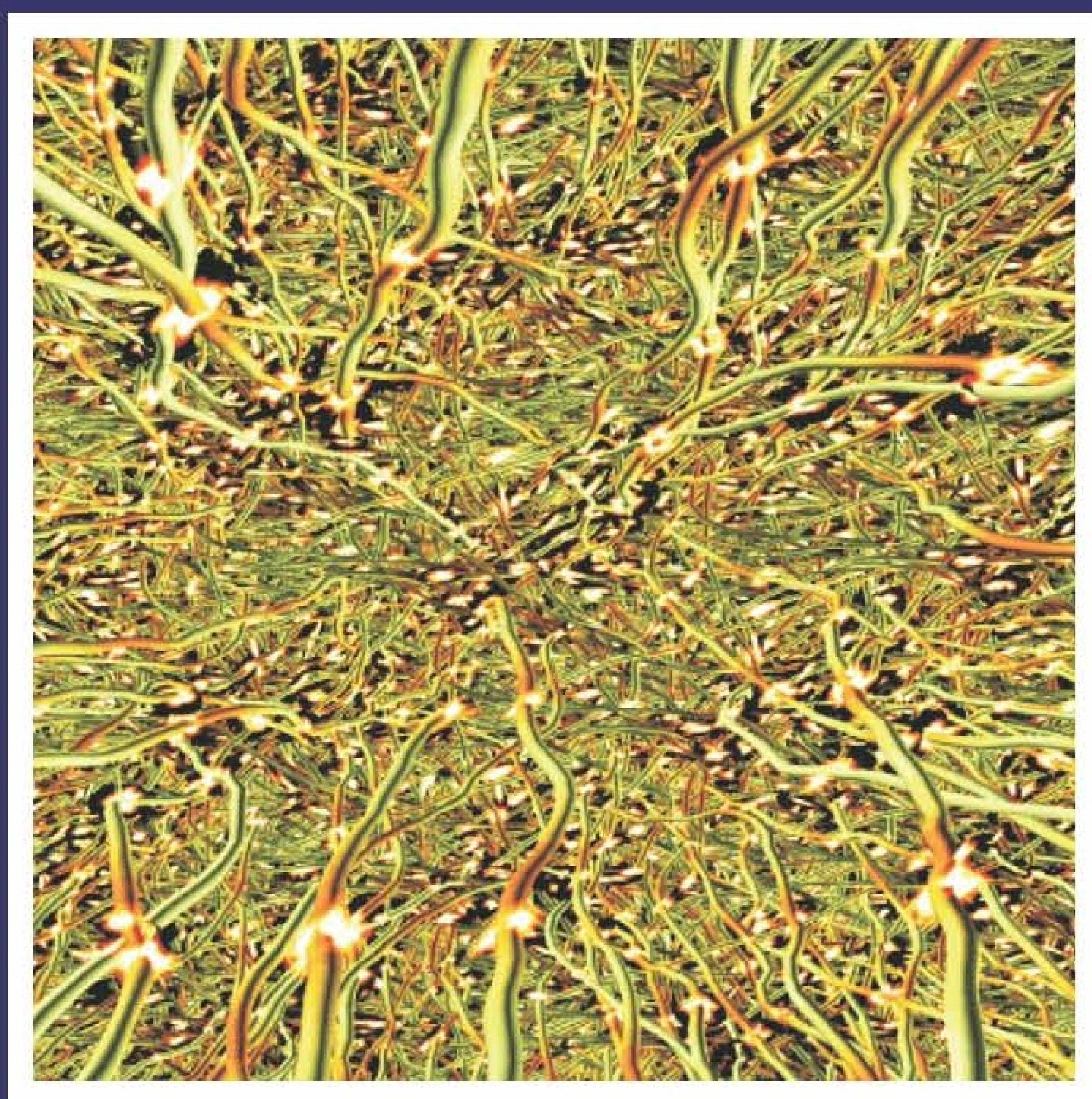

Effects of electrical parameters on the electroencephalogram and physical reflexes of broilers

18

DONDERS series

Waterbath stunning of chickens

Effects of electrical parameters on the electroencephalogram and physical reflexes of broilers

Simone Prinz

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WATERBATH STUNNING OF CHICKENS

EFFECTS OF ELECTRICAL PARAMETERS ON THE ELECTROENCEPHALOGRAM AND PHYSICAL REFLEXES OF BROILERS

Een wetenschappelijke proeve op het gebied van de
Sociale Wetenschappen

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. mr. S.C.J.J Kortmann,
volgens besluit van het college van decanen
in het openbaar te verdedigen op donderdag 11 juni 2009
om 11.00 uur precies

door

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Prof. dr. D. Fletcher (University of Connecticut, USA)

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an academic essay in Social Sciences

Doctoral Thesis

to obtain the degree of doctor
from Radboud University Nijmegen
on the authority of the rector magnificus prof. dr. S.C.J.J. Kortmann, according
to the decision of the council of deans to be defended in public
on Thursday, June 11, 2009 at 11.00 hours

by

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ABSTRACT

Animals must be rendered unconscious before slaughter takes place and effective stunning is a prerequisite for the maintenance of high welfare standards in commercial slaughterhouses. Electrical waterbath stunning is the most common method utilised for broiler chickens but, as the electrical current passes through the head and also the body of the birds, it affects meat quality. High currents may result in muscle convulsions, which can cause blood spots and broken bones. Different electrical waveforms and frequencies are used in a variety of combinations in the poultry industry in order to minimise these defects, but their effectiveness for inducing unconsciousness is not well understood. This thesis deals with the assessment of the electroencephalogram (EEG) of broilers subjected to a range of electrical stunning parameters to determine their effectiveness for the induction of unconsciousness. A non-invasive device to record the broiler EEG was developed to analyse the brain activity following the different stunning treatments. The experimental work revealed that electrical frequency has a major influence on stunning efficiency and that frequencies above 400 Hz were not effective with the maximum tested current of 150 mA. Both rectangular AC and pulsed DC electrical waveforms were equally effective but the duration of the current application when using pulsed DC waveforms appeared to be especially important for stunning effectiveness. In constant voltage waterbaths, female broilers receive a lower stunning current compared to males due to their significantly higher electrical resistance, resulting in a lower stunning efficiency. A two-phase stunner commonly used in the U.S, which delivers a high frequency pulsed DC waveform followed by 50 Hz AC, was also tested. It appeared that this stunning method would require higher currents than those achieved using the 60V electrical supply in this study to achieve adequate stunning results. The effect of the different electrical stunning parameters on meat quality was not assessed in this project and should be investigated in future research. The stunning of broilers using waterbaths requires a good understanding of the different electrical parameters and their effectiveness. However, if appropriate parameters are selected with welfare criteria in mind, it is a viable stunning method for commercial slaughterhouses.

Chapter 1

General Introduction

Background and principle

The legislation of the European Union demands stunning of animals before slaughter (Council Directive 93/119/EC[1]). At present electrical waterbath stunning is the standard method used in commercial chicken slaughterhouses to render the birds unconscious. With this method, the birds' feet are fixed in grounded metal shackles. Hanging upside down, the birds are then moved towards an electrical waterbath. When the animals' heads touch the water the circuit is closed, causing the current to run through the head and body. This leads to a disruption of the depolarised state of the neurones in the brain and thus induces unconsciousness (Raj and Tserveni-Gousi, 2000).

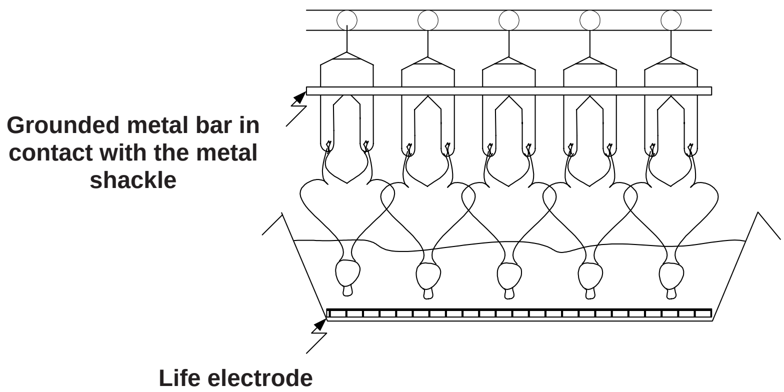

Figure 1: Electrical waterbath (Drawing by E.J. van de Griend)

Low frequency stunning

If the electrical current is too low for an immediate induction of unconsciousness, this may cause pain (von Wenzlawowicz and von Holleben, 2001). Several studies have been conducted to determine the minimum necessary current to render birds unconscious, using the encephalogram (EEG) and physical reflexes. Most of the recommendations are related to a sinusoidal AC waveform of 50 Hz. Results of Gregory and Wotton (1990) show that a minimum of 120 mA prevents recovery of somatosensory evoked potentials (SEP), indicating an unequivocal stun. In the same study a current of 105 mA provided a period of 52 seconds of apparent insensibility, judged by the return of neck tension. Application of 148 mA induces cardiac arrest in at least 99% of the animals (Gregory and Wotton, 1987).Wormuth et al.

[1]http://eur-lex.europa.eu

(1981) concluded that a minimum of 120 mA sine wave AC of 50 Hz causes cardiac fibrillation in 100% of the broilers. This was found to be the quickest method to induce brain failure (Gregory, 1989). For welfare reasons, some authorities therefore recommend a minimum stunning current of 120 mA (Gregory, 1998).

Altering frequency and waveform – an attempt to improve meat quality

Application of high currents on the other hand causes a number of meat quality defects. Gregory and Wilkins (1989) found an increase of breast muscle haemorrhages in broilers stunned with currents higher than 130 mA. Hillebrand et al. (1995) found similar effects in broilers stunned with 100 mA. A high voltage level has been associated with a lower bleed-out (Veerkamp and de Vries, 1983). Red wing tips could be observed with a 50 Hz sine wave AC above 110 mA (Gregory and Wilkins, 1989). In addition, the occurrence of broken wishbones increases with higher voltage levels (Gregory and Wilkins, 1990). Electrical setups have therefore been sought that prevent meat quality defects. This has mainly been achieved by altering the frequency and waveforms of the current. Raj et al. (2001) found a significantly lower occurrence of broken bones and breast meat haemorrhages in broilers stunned with a sine wave AC of 1500 Hz compared to 50 Hz. Gregory et al. (1991) on the other hand could not find a difference in the occurrence of haemorrhages in broilers stunned with 50 or 350 Hz pulsed DC. The incidence of ventricular fibrillation however, was markedly decreased with the higher frequency. It has been shown that pulsed DC stunning has a lower effect on heart function and results in overall better bleed-out (Kuenzel and Ingling, 1977).

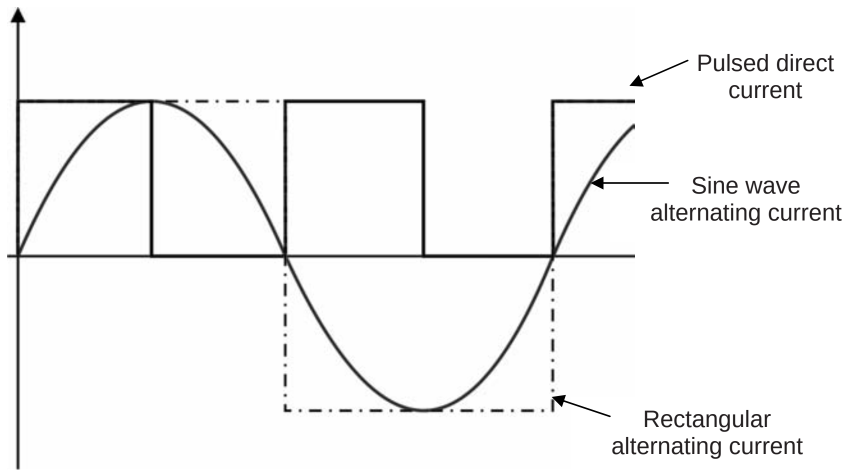

Figure 2. Illustration of different electrical waveforms

Welfare implications of alternative electrical setups

Stunning effectiveness of these alternative setups has not been analysed systematically. If the animals are able to recover from stunning, it must however been assured that the birds remain unconscious until death from bleeding. A minimum of 40 seconds unconsciousness has therefore been suggested (Raj, 2006). Based on the absence of SEPs, a minimum of 120 mA pulsed DC of 350 Hz has been recommended to achieve effective stunning (Gregory and Wotton, 1991). In more recent studies the effectiveness of AC and pulsed DC for the induction of unconsciousness in broilers has been analysed after application in the waterbath for one second (Raj et al., 2006a,b,c). It was concluded that a minimum of 100, 150 and 200 mA must be applied for AC stunning of broilers with up to 200, 400 and 800 Hz AC respectively. Pulsed DC stunning on the other hand has been actively discouraged (Raj et al., 2006b,c)

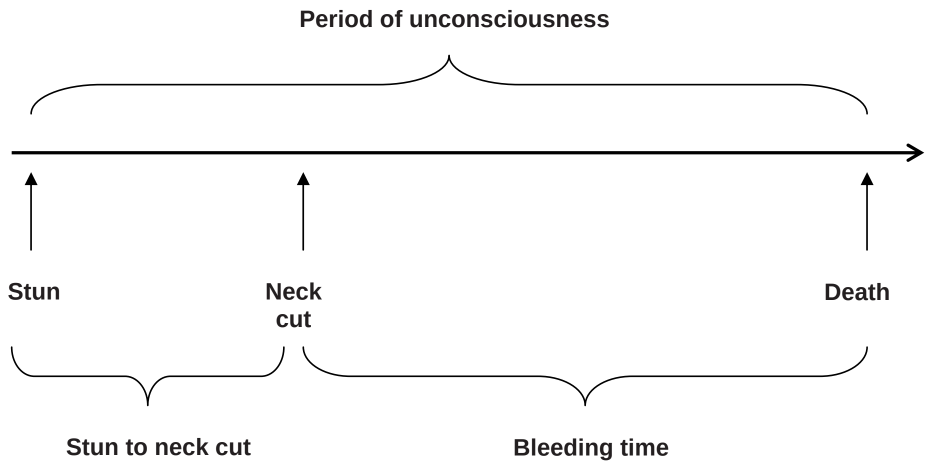

Figure 2: Process flow of electrical waterbath stunning. If the applied electricity does not cause cardiac arrest (stun-to-kill), a sufficient period of unconsciousness must be ensured to prevent recovery of the animals during bleeding. (Drawing adapted from Raj, 2006)

Several investigations have been conducted on different waveforms and frequencies (Wilkins et al., 1998; Wotton and Wilkins 1999) but only physical parameters such as breathing and neck muscle tension were assessed (von Wenzlawowicz and von Holleben, 2001). The reliability of these physical parameters for the assessment of unconsciousness is not well established. Systematic evaluation of several physical parameters in comparison to EEG analysis could indeed provide valuable information to slaughterhouses for the assessment of stunning effectiveness in daily practice.

Quantitative EEG analysis to analyse unconsciousness

In more recent studies, the effectiveness of AC and DC stunning has been analysed using quantitative EEG analysis rather than subjective assessment of brain waves. This provides a better description of the degree of changes in the power content of EEG signals, hence more reliable information on the state of (un)consciousness (Raj and O'Callaghan, 2004a). The occurrence of an iso-electric or profoundly suppressed EEG with less than 10% of the pre-stun EEG power content was used to determine the depth of unconsciousness (Raj and O'Callaghan, 2004a,b; Raj et al., 2006a,b,c). A reduction of total brain power (2-30 Hz band) to less than 10% of the pre-stun brain power indicates the occurrence of an iso-electric or profoundly suppressed EEG. Birds can be assumed to be unconscious provided that a period of at least 30 seconds of iso-electric EEG follows an epileptic fit induced by electrical stunning (Schütt-Abraham et al., 1983). The activity in the relative brain power spectrum (13-30 Hz band) was used as a measure of loss of specific information processing ability in the brain (Raj and O'Callaghan, 2004b). A reduction to less than 10% of the pre-stun level was interpreted with an unequivocal loss of sensibility (Raj et al., 2006a). Based on these criteria, it has been concluded that effective waterbath stunning can be achieved with a sine wave AC of 100, 150 and 200 mA root mean square with maximum frequencies of up to 200, 600 and 800 Hz respectively (Raj et al., 2006a). Pulsed DC stunning on the other hand, required 200 mA average current for a maximum of 200 Hz with a mark:space ration of 1:1 to induce epileptiform activity in 80% of the broilers, while higher frequencies would require higher current levels (Raj et al., 2006b). It has moreover been questioned if pulsed DC stunning is acceptable, as some broilers obtained cardiac arrest without expressing epileptic activity in the EEG (Raj et al., 2006b,c). In both studies the stunning current has been applied for one second (Raj et al., 2006a,b,c). This does not correspond with practice in commercial slaughterhouses, where the current is usually applied for at least four and often for more than 10 seconds.

Waterbath stunning in Europe and the U.S.

Electrical setups applied in chicken slaughter plants consist of a variety of waveform, frequency and current combinations. Both, AC and DC waveforms are applied with frequencies ranging from 50 Hz to 2000 Hz. The average current per bird is usually set between 70 mA and 150 mA in European slaughter plants, but due to the individual impedance of the animals the actual level varies. It has moreover been suggested that the effective current per broiler varies between males and females due to the higher electrical resistance of female broilers (Rawles et al., 1995).The effectiveness of the different setups for the induction of unconsciousness is not well understood (Wilkins et al. 1998).

In the U.S. current levels are considerably lower (25 to 45 mA) compared to the EU (Bilgili, 1999). A step-up stunner (Simmons Engineering Company, Dallas, GA, USA) has been introduced, consisting of two consecutive stunning phases. In Phase I a pulsed DC of 550 Hz is applied with a low voltage of 12-15 V in a shallow brine waterbath. The water depth is approximately 1 cm, with the chickens' head resting on a metal grid. Conductivity in the waterbath is controlled by a "Salt Injector Assembly". This is immediately followed by Phase II, consisting of a metal plate. In Phase II a sine wave AC of 50 Hz is applied with 20-40 V. In the first phase, chickens are rendered unconscious and the body is relaxed, while the second phase induces a deeper stun. This stunning system is now also used in the European Union, but stunning effectiveness of the low currents has been questioned (Raj, 2003). The low voltage in Phase I resulted in adequate stunning effectiveness, judged by the return of rhythmic breathing and tension of the neck muscle (Wotton and Wilkins, 1999). Analysis of EEG signals however, has not been conducted with this stunning method under European conditions.

Aim and outline of the thesis

The aim of the present study is to determine stunning effectiveness of a wide range of electrical setups applied in an electrical waterbath under the same experimental conditions. The influence of the different parameters waveform, frequency and current level and their interrelation is systematically investigated. The effectiveness of the Simmons step-up stunner is analysed in comparison to high voltage stunning in the European Union. Stunning effectiveness is assessed using EEG analysis and evaluation of physical reflexes. The viability of physical reflexes such as corneal reflex, spontaneous eye blinking, breathing and wing flapping for the evaluation of stunning efficiency under commercial conditions is investigated. All aspects are analysed for male and female broilers separately.

Firstly, the development of a non-invasive device for the recording of brain waves, the 'chicken EEG clamp - CHEC' (Coenen et al., 2007) is presented. The second chapter deals with the validation of the CHEC following electrical waterbath stunning and a typical base-line EEG of a broiler chicken is established (Prinz et al., 2009a). In chapter three the influence of amount and frequency of a square wave AC on the induction of unconsciousness is evaluated with currents ranging from 60 to 150 mA and frequencies ranging from 70 to 1500 Hz (Prinz et al., 2009b unpublished). Chapter four describes the influence of the same frequencies of a pulsed DC on the induction of unconsciousness with currents ranging from 80 to 150 mA (Prinz et al., 2009c, unpublished). Chapter four compares the effects of different waveforms, sine wave AC, rectangular AC and pulsed DC at constant voltage levels, on the induction of unconsciousness in male and female broilers (Prinz et al., 2009d, unpublished). Finally, the effectiveness of the Simmons step-up stunner is investigated (Prinz et al., 2009e, unpublished). All results are ultimately compared in a general discussion and the main conclusions are presented.

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Chapter 2

A non-invasive technique for measuring the electroencephalogram of broiler chickens in a fast way: the 'chicken EEG clamp' (CHEC)

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ABSTRACT

A device was developed to measure in a fast way the electroencephalogram (EEG) of broiler chickens in a non-invasive way. The ‘chicken EEG clamp’ (CHEC) consists of a framework with two pointed electrodes, fitting as a clamp around the chicken’s head. The EEG is recorded by the two active electrodes firmly contacting the skin overlying over the midst of the brain. The device is equipped with a pre-amplifier and is grounded. Validation of he CHEC was done in three groups of broilers: 1. chickens anaesthetised with ketamine-xylazine, 2. chickens anaesthetised with carbon dioxide in oxygen, 3. chickens locally anaesthetised with lidocaine applied under the skin of the brain.

It appeared that the EEG can be obtained almost immediately, after 5 to 10 seconds recordings were available. The EEG traces obtained with the CHEC device were considered as reliable for two reasons: EEG patterns of the three groups are different from each other, while the EEG characteristics of the three groups are representative for the anaesthetic used. Even clear EEG spindling, a typical EEG pattern in wake chickens, could be seen in the locally anaesthetised animals. The experiment proved that reliable, external, non-invasive EEG recording of broilers can be obtained in a fast way, allowing quick assessment of the EEG in large numbers of animals.

Keywords

Broiler, electroencephalogram, technique

INTRODUCTION

According to the Council Directive 93/199/EC of the European Union, animals have to be stunned before slaughter. Stunning in an electrical water bath is a technique which is usually applied in commercial chicken slaughterhouses. The birds pass through an electrified water bath while hanging upside down on metal shackles which are connected to ground. A current flows through the body of the chickens when the bird's head touches the electrified water, producing stunning. A variety of electrical parameters are used to adjust the stunning setup in slaughterhouses, including waveform, frequency, voltage as well as stunning time. This leads to variations in stunning efficiency, with some setups causing cardiac arrest, while in others the chickens are able to recover (Wilkins et al., 1998). If the birds do not encounter cardiac arrest, they must remain in a state of unconsciousness until their death from bleeding (Council Directive 93/199/EC).

For animal welfare reasons it is important to assess the reliability of stunning procedures. It is generally known that testing of reflexes only is not a reliable estimate of consciousness in birds. The recording of brain waves by an electroencephalogram (EEG), as an indicator of vigilance and consciousness, has been used (Raj, 2004). Commonly, electrodes are implanted into the chicken's skull, requiring surgery and adequate recovery time. This traditional method is costly and time consuming, and is therefore not suitable for large amounts of animals. D. Fletcher from the University of Georgia (personal communication) tried a method using plasters fitted with electrodes, which were applied to the chicken's head before entering the water bath. However, the method requires extensive handling of the birds before stunning, as the feathers on the chickens' head have to be plucked before application of the electrodes to ensure good contact, causing additional stress to the animals.

In order to appraise the reliability of the existing electrical stunning setups and to further improve stunning devices it is essential to record large numbers of birds within a short time. Therefore a method is sought, allowing fixating external EEG electrodes after electrical stunning without delay and with adequate accuracy. The method should also be applicable under the conditions of commercial slaughter plants.

NON-INVASIVE EEG DEVICE

The device is shown in Figure 1. It is fitted with pointed electrodes opposite to a grounded metal rail. The differential recording electrodes, insulated except the tips, are equipped with a pre-amplifier, transferring the EEG signals from an artefact sensitive high impedance circuitry to a low impedance circuitry, avoiding disturbance of the signal. The chicken's neck is placed into the rail, and the electrode tips are set on the head behind the chicken's comb, on both sides of the brain. Springs ensure tight and stable contact of the electrodes to the skin. To simulate conditions similar to water bath stunning, the bird's head is wetted with water before application of the electrodes in all validation experiments, perhaps even contributing to a good contact between skin and electrodes. The delay in recording of the EEG is minimised to a few seconds due to quick and standardised application of the device. After the electrodes have been fitted to the skin, a button on the device is pressed to discharge the filters in order to obtain a quick EEG signal. This method allows EEG recording within 5 seconds after the broilers have left the stunning bath.

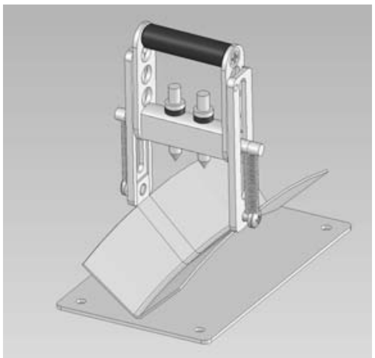

Figure 1. The basics of the non-invasive EEG device, the 'chicken EEG clamp' (CHEC), without cables and amplifiers. The bird's head is moved in the metal rail when the electrode rack is lifted. When the head is in a proper position, the electrode rack is moved down till the electrodes make tight and stable contact with the bird's head. See also the text. (Drawing by Norbert Hermesdorf).

VALIDATION OF THE DEVICE

The Animal Experimental Committee (DEC) of the Radboud University Nijmegen permitted consent for the validation experiment under number RU-DEC 2005-136. In order to evaluate the quality of the EEG signals recorded with the non-invasive EEG device, birds were measured in three conditions. In the first condition birds were measured under ketamine-xylazine anaesthesia, in the second under carbon dioxide anaesthesia, while in the third condition locally anaesthetised birds were registered. In all three conditions 10 commercially available, 5 week old broiler chickens (Ross) were used, both males and females. The average weight was 2.2 and 1.6 kg respectively. The device was fitted to the head of the birds as described above. The recordings included signals from 1-100 Hz, as well as from the 10-100 Hz band. The latter frequency trace is faster visible on the screen of the EEG monitor, due to filtering of the offset voltage. A 50 Hz notch filter prevented noise from the electrical net.

In the first condition animals were intramuscularly injected with a mixture of 0.2 mg/kg ketamin and 0.5 mg/kg xylazine. When the birds showed adequate anaesthesia the EEG of animals was recorded. Representative EEG traces of two animals are shown in the upper panel of Figure 2. In the ketamine-xylazine condition the EEG shows a stereotyped rhythmical burst-like activity followed by a relatively iso-electric pause, in a 1 to 2 Hz periodicity. This EEG pattern is characteristic for a type of anaesthesia induced by xylazine and non-specific NMDA antagonists, such as ketamine. The burst-like activity is caused by neuronal spiking induced by the NMDA antagonists (Soltesz and Deschenes, 1993). After recording the EEG, chickens were euthanized by an injection of an overdose of pentobarbital into the wing vein.

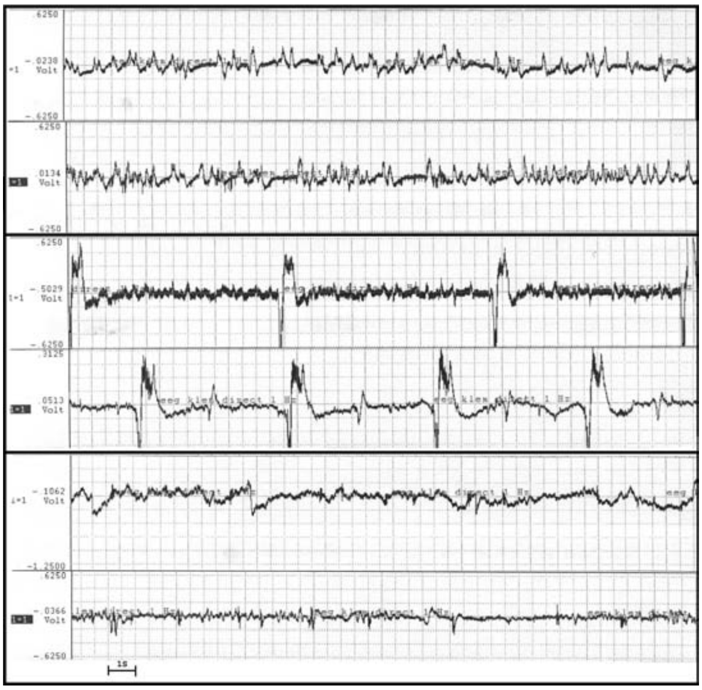

Figure 2. EEG records (1–100 Hz.) of three groups of broiler chickens registered by the chicken EEG clamp (CHEC). The two records in the upper panel are from two animals under ketamine-xylazine anaesthesia, the middle panel shows EEG records of two animals anaesthetised with carbon dioxide, while in the lower panel records are obtained from two locally anaesthetised broilers. See text for further explanations.

A carbon dioxide anaesthesia was induced in the second condition. Birds were placed in a box containing a mix of 50% of carbon dioxide and 50% of oxygen. This mix induces a fast anaesthesia, while animals show deep and slow breathings. The middle panel of Figure 2 displays the carbon dioxide EEG pattern registered by the chicken EEG clamp. The relatively high frequencies are typical for carbon dioxide anaesthesia (Coenen et al., 2000). The periodic variations in the wave-amplitude are smaller as compared to the wave variability in the ketamine-xylazine condition. The slow and deep breathings, occurring every five to eight seconds, are clearly shown in the EEG as movement artefacts superimposed on the low amplitude EEG. Animals were offered after the experiment by reducing the oxygen in the gas mixture.

Birds of the third group received a local anaesthesia with lidocaine under the skin of the entire skull. Animals were further restrained by immobilisation of wings and body. Their EEG was recorded and is presented in the lower panel of Figure 2. Signals are typical for wakeful birds, generally with small amplitude high frequency waves with a higher variability and some more movement artefacts than the anaesthesia wave patterns (Coenen et al., 2006). EEG traces are almost identical to those recorded with the traditional method by implanting permanent electrodes. Also spindling, so characteristic for the bird's EEG in particular over the frontal cortex, is sometimes clearly visible, for example in the lowest trace of Figure 2.

CONCLUSION

Basically there are two strong arguments to consider the EEGs recorded with the non-invasive device, the 'chicken EEG clamp' (CHEC) as genuine EEGs and not as artefacts. The first argument is that the three conditions are associated with EEG patterns which can easily be distinguished from each other. In case the EEG is built up by artefacts not related to the brain state, traces should be more identical. The second argument is that the EEG characteristics of the three conditions are representative for the anaesthetic drug used in these conditions. Birds of the ketamine-xylazine condition show EEG traces that are typical for such anaesthesia, while that is also true for the carbon dioxide condition. One of the most powerful argument stems from the fact that clear spindling could be seen in the records of the birds, registered without complete anaesthesia. In conclusion, the experiment proved that the non-invasive technique for EEG recording allows quick recording of reliable and genuine EEG patterns. Hence, this method allows assessment under EEG guidance of the reliability of stunning in large numbers of birds under commercial slaughter house conditions.

ACKNOWLEDGEMENT

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Chapter 3

The electroencephalogram of broilers before and after DC and AC electrical stunning

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ABSTRACT

Stunning efficiency was established by measuring the electroencephalogram (EEG) of chickens just before and just after electrical waterbath stunning. The chicken EEG clamp (CHEC) was used to record the pre-stun EEG in 14 wake broilers and with Fast Fourier Transformations (FFTs) the total electrical power of this raw base-line EEG was obtained. A grand average FFT showed the typical spectral characteristics of the EEG of a wake bird. Then, animals were divided into three groups: the first group was stunned in a water bath with pulsed DC of 100 V, 70 Hz, the second with pulsed DC of 100 V, 1500 Hz, while the third group was stunned with AC of 100 V 1500 Hz. The reduction of the power in the post-stun EEG, recorded within a few seconds after stunning, was calculated and expressed as a percentage of the average power in the pre-stun EEG as obtained from the grand average of the wake birds. According to literature, a post-stun reduction in the 2-30 Hz band to less than 10% of pre-stun levels expresses iso-electricity and deep unconsciousness, whereas a reduction in the 13-30 Hz band to less than 10% of pre-stun levels might indicate a loss of sensibility. In the 2-30 Hz band only birds of the first group (pulsed DC, 100 V, 70 Hz) showed a profoundly suppressed EEG with less than 10% of the pre-stun EEG power. Broilers of the two other groups (pulsed DC and AC, 100 V, 1500 Hz) showed on the average power contents slightly higher than 10%. Results of the 13-30 Hz generally corresponded with the 2-30 Hz band. In conclusion it could be demonstrated that the chicken EEG clamp (CHEC) is a fast and appropriate method to record the EEG. The EEG of an average wake broiler chicken is a useful tool to compare reduction of EEG power after different electrical stunning setups. It could be established that stunning with 70 Hz pulsed DC is more effective than stunning frequencies of 1500 Hz using a pulsed DC or AC current. Future research with more and smaller frequency intervals is necessary to understand the influence of electrical setups on stunning efficiency.

Keywords

Broiler, electrical stunning, DC and AC electrical setups, unconsciousness, EEG

INTRODUCTION

Electrical water bath stunning is a common method used in commercial chicken slaughter houses. Birds are rendered unconsciousness as their heads are immersed into an electrified water bath while the shackled feet have contact to ground electrodes, causing the current to run through the chickens' head and body. Stunning efficiency varies with different electrical setups, including electrical waveforms, frequencies, currents and voltages. While birds encounter cardiac arrest and death with particular setups, they are able to recover with others. It must be avoided that the chickens regain consciousness before their death from bleeding. Therefore, assessment of stunning efficiency is important to maintain welfare standards during commercial slaughter. Recording of brain waves in the form of an electroencephalogram (EEG) offers a unique opportunity to assess brain vigilance, including unconsciousness, in an objective way (Coenen, 1995; Raj and O'Callaghan, 2004a and 2004b; Schütt-Abraham, 1983).

It is generally accepted that establishing an iso-electric EEG pattern after stunning is characteristic for unconsciousness (Raj and O'Callaghan, 2004; Coenen et al., 2000). Schütt-Abraham et al. (1983) recommends a minimum of 30 seconds iso-electricity as a parameter for adequate stunning. Following this assumption, Raj and O'Callaghan (2004a and 2004b) calculated the total power content of EEG patterns derived from stunned broiler chickens to establish the state of vigilance and consciousness. A total power content of less than 10% of the pre-stun level is considered characteristic for an iso-electric or profoundly suppressed EEG in animals (Raj and O'Callaghan, 2004a and 2004b). To analyse iso-electricity the frequency band of 2-30 Hz in the electrical brain power is used, representing the most relevant information regarding brain functioning. On the other hand the lack of activity in a smaller band from 13-30 Hz in stunned chickens has been interpreted as an 'unequivocal loss of sensibility' (Raj and O'Callaghan, 2004a and 2004b).

In a previous paper the 'chicken EEG clamp' (CHEC) has been introduced to measure the EEG in a fast way and it has been shown that this is a valid method to record brain waves from broiler chickens (Coenen et al., 2007). As the electrodes are pressed on the chickens' head, the laborious implantation of electrodes, associated with additional inconvenience and stress for the birds, can be avoided. The non-invasive method of EEG recording allows a fast visualization of the brain waves of a broad range of broilers. It provides therefore the opportunity to assess the specific stunning consequences in several EEG frequency bands in comparison to pre-stun brain activity. The rationale of this study was twofold. Firstly, the EEG of normal wake broilers was measured, just before stunning, and the characteristics of the EEG were studied in order to construct a typical power spectrum of non-stunned animals. Secondly, the effects of electrical stunning on the EEG were evaluated, using high and low frequency AC and DC currents. This allowed a comparison of the EEG pre- and post-stun, in order to study the effects of stunning on the characteristics and the power spectrum of the EEG.

MATERIAL AND METHODS

The Animal Experimental Committee of the Radboud University Nijmegen permitted consent for this experiment under number RU-DEC 2005-136. Fifteen commercially available male and female Ross broiler chickens with weights ranging from 1187 to 2092 g (average 1458 g) were used as experimental subjects, including one animal that died for unknown reasons in an initial stage of the experiment. Before exposing the broilers to stunning, they were restrained by immobilisation of their wings and the baseline EEG of all 14 birds was measured with the CHEC. EEG recordings included the full range from 1 to 100 Hz, although the EEG was analysed and is presented in the 2-30 Hz and 13-30 Hz bands. After recording the baseline EEG, every bird was hung upside down with the feet fixed into a grounded metal shackle, which was attached to a rotary stand. Then the bird's head was immersed, up to the base of the wings, into an electrified water bath filled with salted water. After stunning the rotating stand swung the bird towards the EEG clamp, mounted on an inclined board. The head of the animals was fixed into the EEG clamp, while the feet were still hanging in the shackle. EEG recording started approximately 5 to 10 seconds after stunning. All EEG recordings lasted at least one minute. Afterwards the stunned animals were offered in a box filled with carbon dioxide.

Stunning of the broilers was achieved with a commercial constant voltage stunner (Meyn Quest Cabinet, Meyn Food Processing Technology, Oostzaan, The Netherlands). The current obtained by each animal was measured while the voltage was kept constant to simulate practical conditions. Broilers were randomly assigned to three groups of five animals: the first group (n=5) received in the water bath a pulsed DC current of 100 V and 70 Hz for 5 seconds, the second (n=5) a pulsed DC current of 100 V and 1500 Hz for 10 seconds, both with a constant mark:space ratio of 1:1, while the third group (n=4) received an AC current of 100 V and 1500 Hz, also for 10 seconds. The waveform of the AC current was rectangular. The average current through the animals for the first group was 98 mA (range 70 – 150), for the second group 87 mA (range 85 – 90), and for the third group 82.5 mA (range 60 – 90).

For the analysis of the EEG records Brain Vision Analyser (Brain Products, Zeppelinstrasse 7, 82205 Gilching, Germany www.brainproducts.com) was used. Fast Fourier Transformations (FFTs) were calculated for 5 intervals of 1 second for each bird both pre-stun and post-stun. The grand average of the 5 FFT intervals was constructed per animal for the pre-stun and post-stun periods to determine the representative power content in the 2-30 Hz band and 13-30 Hz band. The difference in power between the pre-stun and the post-stun value was then calculated. A grand average of the pre-stun FFTs of all wake birds was used to establish the baseline power content of an average wake broiler chicken. In addition three grand averages were calculated for the FFTs of the stunned EEGs in the different groups. The reduction in power content in the three groups after stunning was then calculated in relation to the average wake power content.

RESULTS

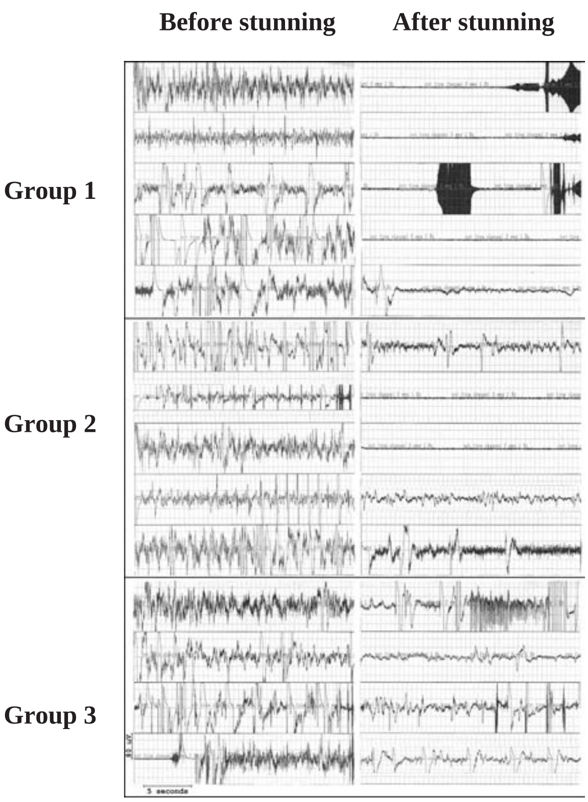

Figure 1. Samples of the raw EEG patterns of the broilers before (left panel) and immediately after stunning (right panel).
In the upper box animals of group 1 (n=5) (pulsed DC 100 V and 70 Hz) are shown (notice the artefacts in some traces), in the middle box those of group 2 (n=5) (pulsed DC 100 V and 1500 Hz) and in the lower box animals of group 3 (n=4) (AC 100 V and 1500 Hz).

Samples of 20 seconds of the raw EEG recordings in the three groups, before and immediately after stunning are presented in Figure 1. A clear reduction of the EEG amplitude as a result of stunning is clearly visible in all groups. However, the level of EEG power reduction differs between the three groups, as well as the number of birds with an iso-electric EEG pattern. After stunning four out of five birds of group 1 (DC 70 Hz) showed an iso-electric EEG. The periods with high amplitudes and high frequencies are artefacts. Only two out of five animals of the second group (DC 1500 Hz) and none of four animals in the third group (AC 100 V 1500 Hz) were iso-electric. One bird of the latter showed a spike-wave epileptic EEG.

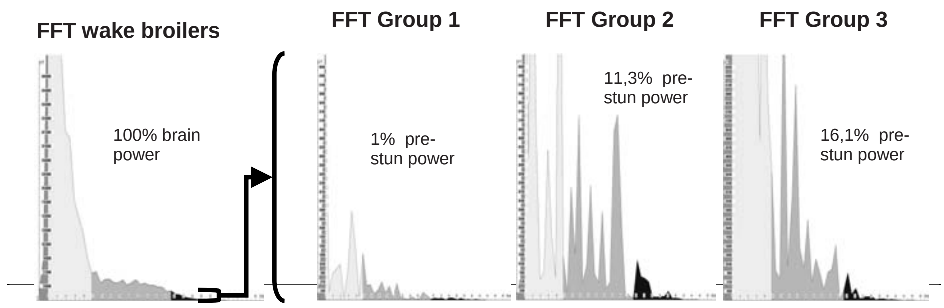

Figure 2. The 'grand average' FFT of the EEG of the broilers just before stunning and of the three stunning groups is shown: Group 1 (DC 100 V, 70 Hz), group 2 (DC 100 V, 1500 Hz) and group 3 (AC 100 V, 1500 Hz). Note the difference in the scale of the Y-axis ranging from 0 to 40,960,000 μV^2 for the wake broilers and from 0 to 665,600 μV^2 in the stunned groups (thus a factor 61 smaller), while the X-axis shows linear frequencies from 2 to 50 Hz.

The 'grand average' of the pre-stun FFT of all animals is presented in Figure 2. It is obvious that the level of vigilance during recording is high. This was due to the fixation of the animals in the EEG clamp. Indeed, from the grand average it can be seen that the high frequency power in the 13 to 30 Hz band, expressing wakefulness, is considerably high, while the low frequencies are still dominating. As expected, stunning induces a sharp reduction of EEG power very obvious from the post-stun FFTs of the three groups in Figure 2. The vertical scale of the post-stun FFTs is by factor 61 smaller than that of the pre-stun FFTs, underlining the dramatic reduction in EEG power.

In the 2-30 Hz band only the chickens stunned with 100 Volts 70 Hz pulsed DC showed a profoundly suppressed EEG with much less than 10% of the average wake FFT. In this group only one animal still showed some minimal brain activity after stunning. Broilers stunned with 100 V 1500 Hz pulsed DC (group 2) and 100 V 1500 Hz AC (group 3) showed power contents slightly higher than 10% of the average wake bird, with 11.3% and 16.1% respectively. In both groups two birds showed still considerable EEG power after stunning, one showed typical epileptic activity expressed in spike-wave discharges. Regarding the results of the 13-30 Hz band the results generally correspond to those of the 2-30 Hz band. Again the animals of group 1 stunned with a pulsed DC current of 70 Hz showed a clearly suppressed EEG with an average reduction in power content to 1%. In the broilers of group 2 the EEG was reduced to about 10% of the pre-stun level. The EEG power in group 3 was lower than 10% in the 13-30 Hz band, but higher in the 2-30 Hz band.

DISCUSSION

The present study had two main objectives. The first was to measure and assess the typical EEG pattern of wake birds with the chicken EEG clamp (CHEC) technique, as developed and described by Coenen et al. (2007). It was indeed possible to obtain reliable EEG records in these animals within approximately 5 seconds and from the Grand Average the typical and representative EEG shown by a broiler just before stunning could be established. The second goal of the present research was to show the direct effects of electrical stunning on the EEG of birds. The adequacy and reliability of the EEG technique can be concluded from the differences in EEG characteristics of the birds of the three stunning groups, contradicting the presumption that the patterns are merely formed by artefacts. In general a sharp reduction of the EEG amplitude was obtained for all stunning groups. Calculation of FFTs pre- and post-stun was used to assess the reduction in total brain power content as a difference to the average pre-stun chicken EEG. Only birds stunned with a low frequency of 70 Hz pulsed DC achieved a reduction of total power content after stunning to less than 10% of the pre-stun level in all cases, indicative for a profoundly suppressed, iso-electric EEG (Raj and O'Callaghan 2004a, 2004b). It is generally agreed that animals with an iso-electric EEG are fully unconscious (Coenen, 1995), and it is convincingly clear that 100 V and 70 Hz induced unconsciousness in all birds. The electrical setups with high frequency of 1500 Hz, both with pulsed DC and AC, did not achieve an adequate reduction in brain power content after stunning in all birds. This is even more significant as the stunning time for the 1500 Hz setups was considerably longer (10 seconds) than used for the DC 70 Hz stunning (5 seconds). Both 1500 Hz setups reduced the power just not under 10% of pre-stun EEG power in the overall frequency band from 2 to 30 Hz, which makes it difficult to judge the state of the animal’s brain exactly. Verification with a higher number of birds and more and smaller frequency intervals in the waterbath is therefore necessary to understand stunning effectiveness with different electrical setups. It can however be assumed that a voltage of 100 V is not sufficient for high frequency stunning with alternating or pulsed direct current. Regarding the 13-30 Hz band which seems indicative for information processing ability (Raj and O’Callaghan 2004a, 2004b), the results generally corresponded with those of the 2-30 Hz band. Future research has to reveal whether the power in the 13-30 Hz band delivers more concise information about unconsciousness and insensibility compared to the more extended frequency band of 2-30 Hz.

CONCLUSIONS

Establishing a pre-stun EEG of an average wake chicken facilitates comparison of reduction in brain power content after electrical water bath stunning. Three stunning set-ups were used: 1. a pulsed DC current of 100 V and 70 Hz, 2. a pulsed DC current of 100 V and 1500 Hz and 3. an AC current of 100V and 1500 Hz. The low frequency stunning with 70 Hz pulsed DC lead to a profound reduction in total brain power content and can therefore be assumed as a viable method to render broiler chickens unconscious before slaughter. Stunning using high frequencies of 1500 Hz seems to be less effective, both in using AC or pulsed DC currents. Further investigation is necessary concerning the influence of electrical setups on stunning efficiency.

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Chapter 4

Electroencephalograms and physical reflexes of broilers after electrical waterbath stunning using an alternating current

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ABSTRACT

Stunning efficiency of broilers following electrical waterbath stunning with an alternating current was assessed using electroencephalograms (EEG) and physical reflexes. 489 broilers (246 males and 243 females) were stunned in an electrical waterbath with a rectangular alternating stunning current of 60, 80, 100, 120 and 150 mA and frequencies of 70, 100, 200, 400, 800 and 1500 Hz. Stunning time was 10 seconds. The EEG was recorded for 120 s post-stun. Occurrence of spontaneous breathing, eye blinking and wing flapping and the corneal reflex were recorded. The EEG was analysed regarding the occurrence of a profound suppression to less than 10% of the pre-stun level in the 2-30 Hz and 13-30 Hz band. The occurrence of epileptiform patterns was assessed and the occurrence of clonic-tonic convulsions was recorded. Statistical analysis showed a highly significant effect of stunning frequency and stunning current for all groups in the EEG analysis. Stunning frequencies above 400 Hz or below 100 mA did not result in profound suppression of brain power to less than 10% of the pre-stun level in more than 90% of the broilers and therefore can not be recommended. More than 80% of the birds stunned with 70 or 100 Hz at 100 mA or 70,100 and 200 Hz with 120 and 150 mA did not recover from stunning. The occurrence of epileptiform activity could only be observed in a few animals. It is assumed that this was due to the long stunning time of 10 seconds and epileptiform activity could have occurred just before the EEG recording started. A maximum of 30% of birds with corneal reflexes and spontaneous eye blinking should not be exceeded, while at 15 seconds post-stun not more than 15% of animals should show spontaneous blinking. Wing flapping occurred in at least 50% of birds with adequate stunning results. This seems to be related to convulsions and could cause meat quality defects.

Keywords

broiler, stunning, rectangular alternating current, electroencephalogram EEG, physical reflex

INTRODUCTION

Electrical waterbath stunning is the most commonly used method in commercial chicken slaughterhouses to render birds unconscious before slaughter. The electricity applied has an influence on both stunning effectiveness and meat quality. To optimise animal welfare and product quality, commercial chicken slaughterhouses apply a variety of electrical setups differing in type and amount of current, electrical frequency and stunning time. An alternating current is often used, delivering either a sinusoidal or a rectangular waveform. The frequencies range from 50 Hz to 2000 Hz with an average current per bird between 70 and 150 mA in European slaughter plants.

Induction of cardiac arrest during stunning has been discussed beneficial to prevent recovery of the birds (Gregory, 1998). A minimum current of 148 mA at 50 Hz sine wave AC was recommended to achieve ventricular fibrillation in 99% of broilers (Gregory and Wotton, 1987) whereas 120 mA would ensure an unequivocal stun (Gregory and Wotton, 1990). Cardiac fibrillation has on the other hand been associated with muscle convulsions, leading to severe meat quality defects such as broken bones, haemorrhaging and red wing tips (Gregory, 1989). High frequency stunning has therefore been introduced, as this does not cause cardiac fibrillation in the waterbath (Gregory and Wotton, 1991). Clearly, unconsciousness must be ensured for animals that did not fibrillate during stunning for a sufficient period of time to prevent recovery during bleeding (Raj, 2003). Assessment of stunning efficiency is therefore essential to maintain welfare standards, taking into account the range of frequency and current combinations usually used under practical conditions. At present, the effects of the different electrical parameters on the chicken are not well understood.

Under laboratory conditions recording of brain waves through electroencephalograms (EEG) has been used to analyse the state of the chicken's brain following stunning and thus determine the state of the animal and the level of (un)consciousness. In order to avoid the laborious implantation of EEG electrodes the "chicken EEG clamp – CHEC" (Coenen et al., 2007) has been developed as a non-invasive method to record brain waves. This method allows assessment of a greater number of birds with various combinations of electrical stunning parameters, facilitating a systematic investigation of the different stunning setups. The occurrence of an iso-electric, flat EEG, as well as the occurrence of epileptic seizures has been used to indicate unconsciousness in chickens (Schütt-Abraham et al., 1983; Raj and O'Callaghan 2004a, b; Raj et al., 2006a). An iso-electric or profoundly suppressed EEG is generally defined as a flat line with an average brain power content of less than 10% of the wake EEG (Raj and O'Callaghan, 2004a, b; Raj et al., 2006a). This is associated with unconsciousness and the inability to feel pain (Coenen, 1995) Prinz et al. (2009) have analysed the representative brain power content of a wake broiler chicken using the CHEC. Through calculation of the relative post-stun brain power, this allows comparative assessment of stunning quality with different electrical setups. For assessment of brain power, two different frequency bands of the brain have been used: 1. a broad frequency band of 2-30 Hz, representing all states of consciousness, and 2. a smaller band of 13-30 Hz indicating perception of, and sensitivity to stimuli, representing the ability to process information (Raj and O'Callaghan, 2004a, b; Raj et al., 2006a). A reduction in total brain power content to less than 10% of the pre-stun power in the 2-30 Hz band has been interpreted with an overall loss of brain function, whereas the same reduction in the 13-30 Hz band has been used to indicate loss of sensitivity (Raj and O'Callaghan, 2004a, b; Raj et al., 2006a) Epileptic activity usually shows low frequency spike and wave discharges on the EEG recordings (Raj et al., 2006a). Based on these criteria Raj et al. (2006a) concluded that a minimum AC of 100, 150 and 200 mA would be necessary at frequencies of up to 200, 400 and 800 Hz respectively to achieve unconsciousness in broilers.

The assessment of the EEG is the most objective and widespread method to analyse the brain state of an animal and is therefore a valuable method to understand the general influence of different electrical parameters. In daily slaughterhouse practice however, assessment of stunning efficiency is only possible through observation of the animals' physical appearance where behavioural reflexes such as breathing, corneal reflex and neck tension are usually used (von Wenzlawowicz and von Holleben, 2001). The occurrence of tonic-clonic seizures has been associated with the occurrence of epilepsy (Schütt-Abraham et al., 1983). The relation between physical reflexes and the state of the chickens' brain, however, is not well established. An evaluation of the characteristics of physical reflexes together with analysis of unconsciousness on the EEG records would therefore facilitate animal welfare assessment in slaughterhouses.

The aim of this study was twofold: firstly, to investigate the effectiveness of different amounts and frequencies of an alternating current using EEG assessment, taking into consideration the range of setups used in European slaughter plants. Secondly, to establish the relation of several physical parameters under conditions that ensure adequate welfare standards.

MATERIALS AND METHODS

A total of 489 broiler chickens, 246 males and 243 females, were raised in one flock for 7 weeks. Average weight was 2907 ± 271 g for males and 2305 ± 229 g for females. For stunning, the animals' feet were fixed into a grounded metal shackle. Thus, single birds hanging upside down were immersed into an electrified waterbath up to the base of their wings. Before stunning, the feet and shackle were sprayed with water to improve conductivity. The waterbath consisted of a plastic basin filled with water, in which a metal electrode covered the whole bottom. Salt was added to the water to maintain the conductivity at four millisiemens/cm. A commercially available constant voltage stunning cabinet (Quest Cabinet, Meyn Food Processing Technology, Oostzaan, The Netherlands) was used to apply a rectangular AC current at frequencies of 70, 100, 200, 400, 800 and 1500 Hz. Voltage was adjusted to obtain the intended r.m.s. (root mean square) current of 60, 80, 100, 120 and 150 mA. The actual current depends on the electrical resistance of the individual bird and was measured using a scope and current probe (123 Industrial Scope Meter {20 MHz} and 80i-110s AC/DC, Fluke Corp., Everett, USA). All data was recorded on to a data acquisition programme (View SW90W, Fluke Corp.). For subsequent analysis birds were sorted according to the actual current, thus leading to small variations in group size. The average stunning time was 10.3 ± 0.7 seconds. The number of animals per group, average voltage and the average current are given in Table 1. The small group of animals that received a current exceeding 150 mA was not analysed.

To ensure immediate EEG analysis following stunning, the shackle was attached to a rotary stand, which allowed quick transfer of the birds to the CHEC (Coenen et al., 2007). The birds' head was fixed in the chicken EEG clamp, while the feet were still hanging in the shackle. This facilitated EEG recording within 8.9 ± 2.9 seconds post-stun. The occurrence of spontaneous breathing, spontaneous eye blinking and spontaneous wing flapping was assessed and recorded on observation channels on the EEG recording. This facilitates a direct comparison between the brain wave patterns and the behaviour of the animals. In addition, the corneal reflex was tested by touching the cornea with a feather at 20 and 40 seconds post-stun. Neck tension was assessed at 30 seconds post-stun, but due to the fact that the birds were fixed in the clamp, evaluation was difficult and this factor was not included in the analysis. When exiting the waterbath the birds' eyes were observed (open or closed) and tonic or clonic convulsions were recorded. Tonic-clonic convulsions were defined as a rigid backward bending of the neck and tucked wings, sometimes accompanied by small and quick muscular contractions, followed by a relaxation of the body. In clonic-only convulsions vigorous wing flapping or large rhythmic contractions of the legs was observed.

Table 1. Number of animals, average voltage and average current are shown for the different stunning groups.

	Stunning Frequency	**No. of animals**			**Average Voltage**		**Average Current**
	Hz	♂	♀	**Total**	♂ **(V)**	♀ **(V)**	**mA**
60 mA	70	9	8	**17**	53	69	59
	100	9	8	**17**	49	71	58
	200	9	11	**20**	47	67	57
	400	12	11	**23**	53	75	61
	800	11	8	**19**	51	71	61
	1500	8	8	**16**	45	65	57
80 mA	70	9	7	**16**	73	89	81
	100	9	9	**18**	71	93	79
	200	8	9	**17**	72	97	83
	400	7	9	**16**	74	90	79
	800	8	8	**16**	66	91	82
	1500	4	5	**9**	60	88	81
100 mA	70	7	7	**14**	86	103	100
	100	7	7	**14**	83	116	101
	200	8	9	**17**	89	111	99
	400	5	7	**12**	88	103	98
	800	4	6	**10**	89	104	103
	1500	6	9	**15**	78	109	99
120 mA	70	4	7	**11**	90	134	119
	100	6	4	**10**	90	115	118
	200	8	4	**12**	90	119	117
	400	12	8	**20**	95	120	118
	800	10	6	**16**	92	112	118
	1500	5	6	**11**	90	112	120
150 mA	70	5	8	**13**	123	153	150
	100	6	4	**10**	109	155	143
	200	4	6	**10**	121	154	147
	400	0	7	**7**	-	150	147
	800	8	10	**18**	110	142	146
	1500	13	8	**21**	99	139	142
Total		**221**	**224**	**445**			

EEG recordings and all observations lasted for 120 seconds post-stun; birds were afterwards euthanized in a box filled with carbon dioxide. The encephalogram was recorded with a sampling rate of 1000 samples per second for every channel using a data acquisition PC-card (Di400, Dataq Instruments, Inc., Akron, OH. USA. www.dataq.com). A physiological analogue amplifier (designed and built by the Electronic Research Group at Radboud University Nijmegen, Nijmegen, The Netherlands) delivered an amplification of 62500. Signals were filtered with a band pass filter of 1-100 Hz, while the 50 Hz Notch Filter was activated for EEG recordings.

For analysis, EEG recordings were transferred to an EEG analyzer (Brainvision Analyzer, Brain Products, 82205 Gilching, Germany www.brainproducts.com) using a Software-aid to convert Windaq-data (Dataq Instruments, Inc., Akron, OH. USA. www.dataq.com). To evaluate total brain power the recordings were filtered for the wide frequency band of 2-30 Hz and for the more limited frequency band of 13-30 Hz. EEGs of every bird were then subdivided in three post-stun periods, P1 0-20, P2 20-30 and P3 30-40 seconds post-stun. In all three periods five segments of one second were marked and a Fast Fourier Transformation (FFT) was calculated to obtain the total power of every segment. The grand average of all five segments of each period was then calculated as the representative EEG power in that period. This procedure facilitated the analysis of genuine EEG recordings without manipulation, where movement artefacts or disturbances, due to testing of physical reflexes, have impaired the EEG. The representative brain power thus obtained from the grand averages of the five segments in each period was then expressed as a percentage of the representative wake chicken EEG (Prinz et al., 2009). The resulting percentage shows the relative brain power following the different stunning treatments. A percentage below 10% of the wake chicken EEG was considered as profoundly suppressed or iso-electric, while a percentage above 10% of the wake chicken EEG represents failure of profound suppression. Birds with an iso-electric EEG were considered unconscious, while failure of an iso-electric EEG indicated inadequate stunning. This was analysed for both frequency bands, 2-30 Hz and 13-30 Hz.
EEG recordings were also evaluated visually. Epilepsy was marked where the trace showed typical spike and wave pattern with a frequency of 2-6 Hz (Figure 1). A characteristic chaotic EEG pattern with high amplitude and high frequency directly following stunning, followed by an iso-electric EEG could be observed in many birds. This was also regarded as an indicator for a form of unconsciousness.

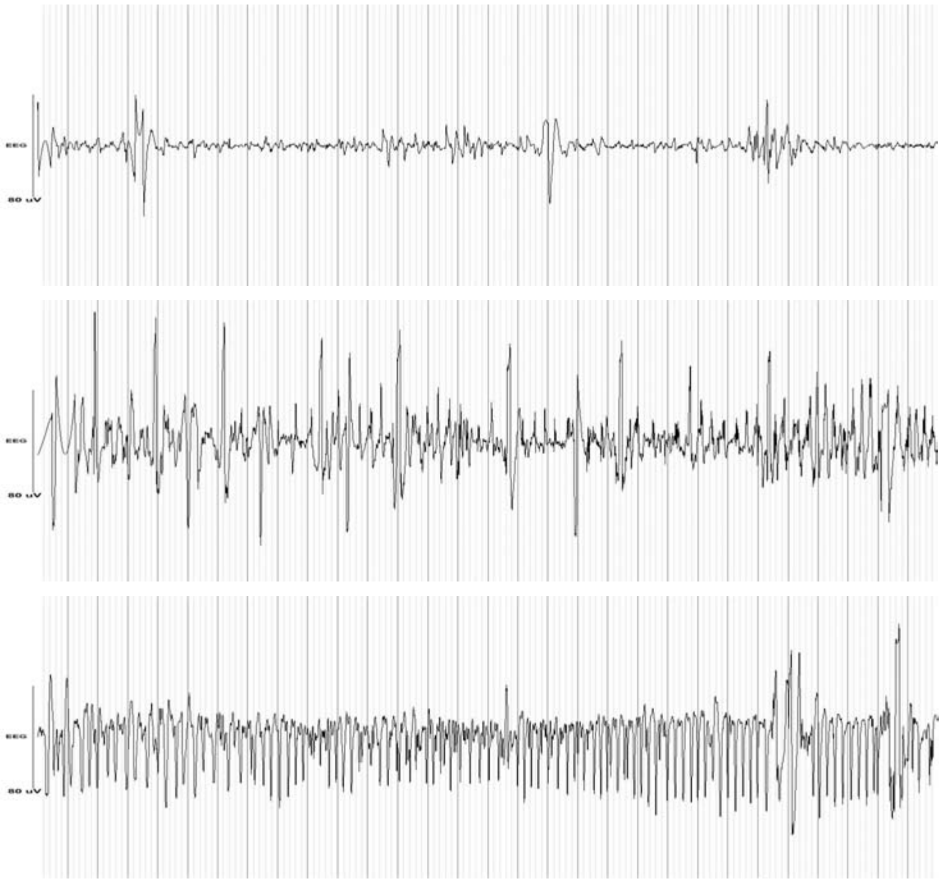


Figure 1. Examples of EEG traces of broiler chickens following waterbath stunning. The upper panel shows a profoundly suppressed, iso-electric EEG with some artefacts caused by the testing of physical reflexes. In the middle panel an EEG without a profound reduction of brain power is displayed. The lower panel contains an example of epileptiform activity with a frequency of 2-6 Hz, with spike-wave discharges. The amplitude of the Y-axis is 80 μV and the area between the bold vertical lines represent 1 second.

For statistical analysis JMP 7.1 (2007) was used. Data was analysed using Nominal Logistic Regression with stunning frequency [Hz], stunning current [mA], the interaction of frequency x current and sex as fixed factors. The factor effects were calculated with the Chi-squared [χ^2] Likelihood Ration Test. Subsequently the probability for EEG responses expressing consciousness or the occurrence of physical reflexes was extracted from the predicted values and plotted.

RESULTS

A total of 418 EEG traces were submitted to Fast Fourier Transformation (214 and 204 for males and females respectively). 71 EEG traces could not be included due to movement artefacts and disturbances. The statistical analysis showed a significant effect of stunning frequency and current on the occurrence of an iso-electric EEG with less than 10% of the pre-stun brain power content ($p<0.0001$) for both EEG frequency bands, 2-30 Hz and 13-30 Hz. From the nominal logistic regression the predicted values were extracted for the percentage of birds that did not show an iso-electric EEG for the different stunning setups. The summed results for the complete time frame 0-40 seconds post-stun are presented in Figure 2.

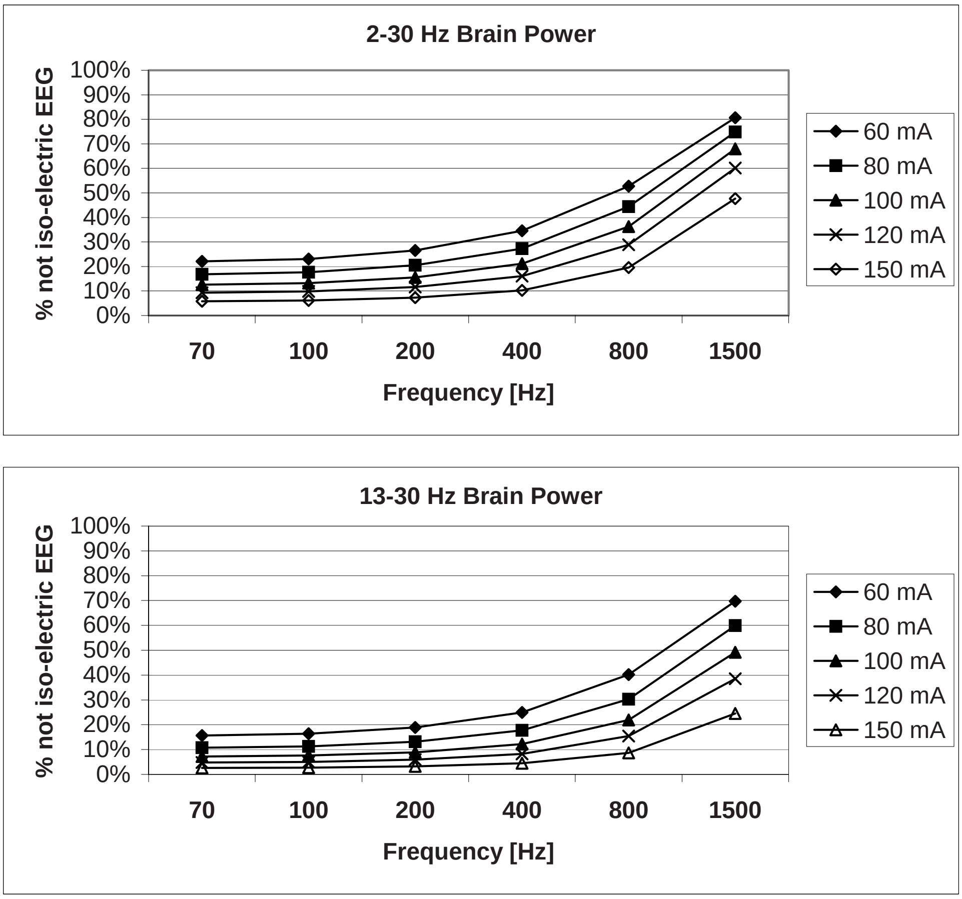


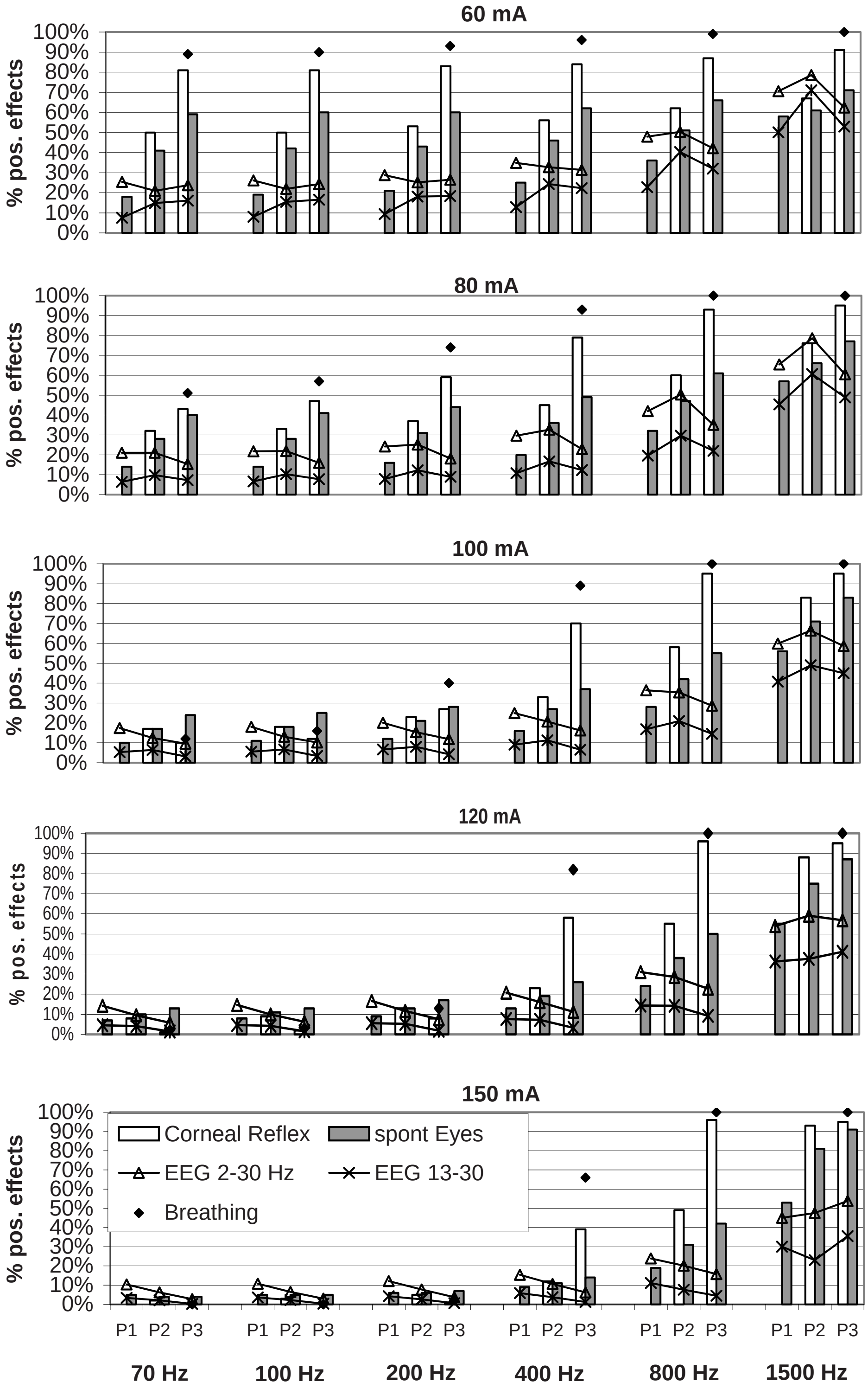
Figure 2. Percentage of birds not showing an iso-electric EEG pattern with less than 10% pre-stun power in the first 40 seconds post-stun, for the broad brain frequency band of 2-30 Hz (upper panel) and for the narrow frequency band of 13-30 Hz (lower panel).

It is obvious that with increasing frequency, a higher amount of current is necessary to achieve an iso-electric EEG, and thus unconsciousness, in an adequate number of birds. In order to achieve a higher stunning efficiency, the amperage must be increased when the frequency is kept constant. The results for the two EEG frequency bands are similar, although for the more limited band of 13-30 Hz, which has been interpreted to indicate sensitivity, lower amperages are sufficient than for the wide band of 2-30 Hz. From the results of the wide band of 2-30 Hz it can be seen that a minimum current of 150 mA must be applied at a frequency of 400 Hz, when 90% of the birds are effectively stunned for 40 seconds after leaving the waterbath. If stunning efficiency is reduced to 85% of birds, the stunning frequency could be increased to 600 Hz or a lower stunning current of 125 mA could then be used at the same stunning frequency of 400 Hz.

The development of the EEG in the three post-stun periods P1 (0-20 seconds), P2 (20-30 seconds) and P3 (30-40 seconds) was analysed with the logistic regression. For all three periods, the effect of stunning frequency was significant ($p<0.0001$) for both brain frequency bands, 2-30 Hz and 13-30 Hz. The effect of stunning current was significant for P2 and P3 ($p<0.0001$) for both frequency bands. In P1 it showed a significant effect in the 2-30 Hz band ($p=0.005$) and was close to the level of significance in the 13-30 Hz band ($p=0.07$). In P3 the interaction of stunning current and stunning frequency also showed a significant effect for both EEG frequencies, 2-30 Hz ($p=0.03$) and 13-30 Hz ($p=0.01$). The predicted values of birds not showing an iso-electric EEG in the three post-stun periods are presented in Figure 3 for all stunning setups.

Assessment of eye reflexes showed similar results as compared to the findings from the EEG. Spontaneous eye blinks showed a significant effect ($p<0.0001$) for stunning frequency in all three post-stun periods. The effect of stunning current was also significant in all three periods ($p=0.0008$; $p<0.0001$; $p<0.0001$ for P1, P2 and P3 respectively), whereas the interaction between stunning current and stunning frequency was significant in P2 and P3 ($p<0.0001$) and close to the level of significance in P1 ($p=0.09$). The results of the predicted values for the occurrence of spontaneous eye blinking in all three periods are presented in Figure 3.

Right page: Figure 3. Percentage of birds not showing an iso-electric EEG (<10% pre-stun power) and percentage of birds with positive behavioural reflexes: corneal reflex, breathing and spontaneous eye blinking in different periods post-stun: P1 0-20 seconds, P2 20-30 seconds, P3 30-40 seconds.


60 mA
80 mA
100 mA
120 mA
150 mA
% pos. effects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Corneal Reflex
spont Eyes
EEG 2-30 Hz
EEG 13-30
Breathing
P1 P2 P3
70 Hz
100 Hz
200 Hz
400 Hz
800 Hz
1500 Hz

The early corneal reflex test at 20 seconds post-stun was significant (P<0.0001) for stunning frequency, stunning current and the interaction of current x frequency. The later test at 40 seconds showed a significant effect (p<0.0001) for stunning frequency and the interaction of frequency x stunning current, but no effect of the stunning current. The predicted values for a positive reflex in both tests are shown in Figure 3 for all stunning setups. The occurrence of breathing within the first 40 seconds post-stun was assessed as an indicator for the survival rate of the chickens for the different stunning setups.

The average time to resumption of breathing was 11.4 ± 3.9 seconds. The statistical analysis regarding the likelihood of birds surviving the stunning process showed a significant effect for stunning frequency and the interaction of stunning frequency x stunning current (p<0.0001). Stunning current did not have a significant effect on the occurrence of breathing within the first 40 seconds post-stun. The predicted percentage of breathing birds in the different stunning groups is given in Figure 3.

Wing flapping was assessed as an indicator for meat quality defects due to bruising or broken bones. The Effect Likelihood Test indicated strong significance (p<0.0001) for stunning current, stunning frequency and the interaction of current x frequency.

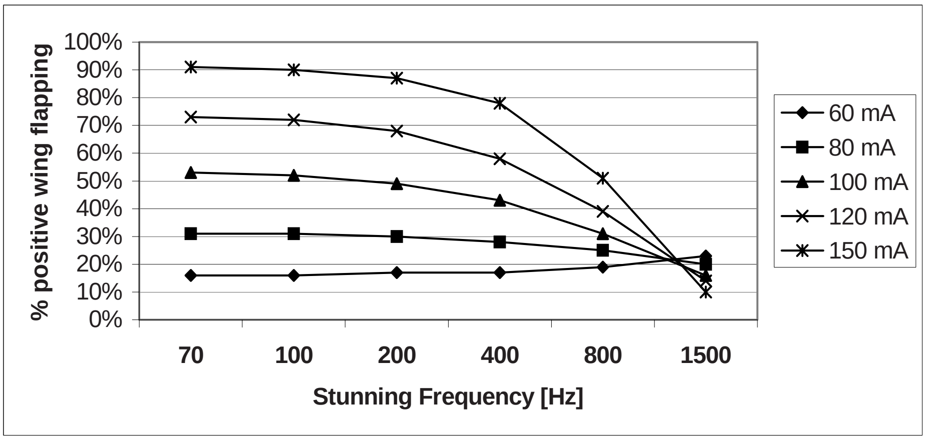


Figure 4. Percentage of wing flapping for different stunning setups.

All parameters were analysed for differences between male and female broilers, but no significant effect could be detected. In the visual analysis a small percentage of epileptiform EEG patterns could be observed. The occurrence of epileptic seizures was significant in the statistical analysis for stunning frequency (p=0.01) and stunning current (p=0.048) but not for the interaction. Epileptic seizures could mainly be observed within the first 30 seconds post-stun (P1 and P2). With higher currents the occurrence of epilepsy decreased and sometimes started between 30-40 seconds post-stun (P3). In groups stunned with currents above 100 mA and low frequencies of 70 Hz no epileptic activity could be observed. The percentage of animals showing a seizure in the EEG pattern with an epileptic fit are summarised in Figure 5. Moreover, the occurrence of tonic-clonic or clonic-only convulsions was assessed for all birds leaving the waterbath. Stunning frequency and stunning current showed a significant effect (p<0.0001) as well as the interaction of frequency x current (p=0.005). Sex also proved to be significant (p=0.0099) with females generally showing more convulsions than males. The results of the tonic-clonic and clonic-only seizures are summarised in Figure 5.

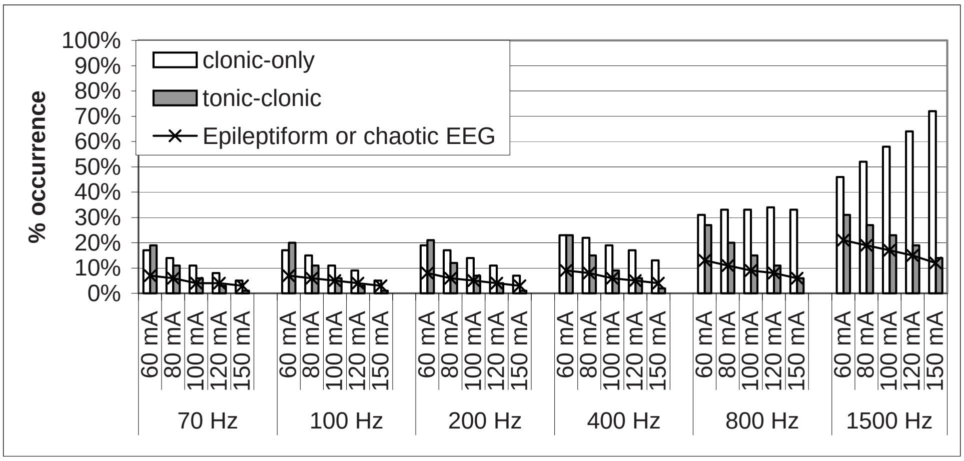


Figure 5. Percentage of animals showing an epileptiform or chaotic EEG (low frequency epilepsy and slow wave disturbance) and tonic-clonic or clonic-only convulsions after exiting the waterbath.

Assessment of the state of the birds' eyes when leaving the waterbath showed a significant effect for stunning current and stunning frequency (p<0.0001) and for the interaction (p=0.0004). Most animals had their eyes open when stunned with high frequencies or with lower frequencies and currents below 100 mA. Almost no bird that was stunned with 120 or 150 mA up to 200 Hz, left the waterbath with open eyes.

DISCUSSION

The first aim of this study was the determination of optimum stunning current and frequency that effectively renders broiler chickens unconscious when using a rectangular alternating current. The electrical setups were selected to represent the range of stunning parameters applied in European slaughter plants. Recent scientific evaluations were also taken into consideration.

Assessment of stunning efficiency using EEG analysis

A profoundly suppressed EEG to less than 10% of the pre-stun brain power has been used as a criterion for unconsciousness in broiler chickens (Raj and O'Callaghan 2004a,b; Raj et al., 2006a). In the present study this could be observed in many birds, with the best results in groups stunned with low frequencies and high amperage. From Figure 2 it is obvious that stunning frequencies above 400 Hz are not suitable to render an adequate amount of birds unconscious for 40 seconds post-stun. Only with 150 mA and a stunning frequency of 400 Hz an overall stunning effectiveness of 90% of the birds could be achieved, while with lower currents the frequency must be decreased. Currents below 120 mA did not appear to be suitable to render 90% of birds unconscious for 40 seconds post-stun. If stunning efficiency was expected to be 85% an AC current of 100mA at low frequencies would be acceptable, whereas lower currents failed to have an acceptable stunning effect on broiler chickens and can therefore not be recommended. These findings correspond with the study of Raj et al. (2006a). Analysis of the smaller frequency band of 13-30 Hz, shows the same trend. However, stunning effectiveness is slightly higher with fewer birds not showing an iso-electric or insensitive EEG pattern (Figure 2). Raj and O'Callaghan (2004b) did not find this difference. In their studies the wide frequency band of 2-30 Hz usually indicates better stunning efficiency than the narrow band of 13-30 Hz. The smaller band only includes higher frequencies, generally associated with wakefulness and vigilance (Coenen, 1995). A profound suppression of this band has been interpreted with the loss of sensitivity in broiler chickens (Raj and O'Callaghan, 2004a, b; Raj et al., 2006a).

EEG and behavioural parameters following stunning

Inadequate stunning setups

Development of the EEG and the behavioural reflexes in the three post-stun periods (Figure 3) clearly show the quick recovery of birds stunned with low stunning currents of 60 and 80 mA. While most birds survived stunning with low currents, the electricity applied did not achieve an adequate period of profoundly suppressed EEG in more than 85 % of the birds, even in the first 20 seconds. Corneal reflexes at 20 seconds post-stun were positive in more than 30% of these birds with all frequencies. This underlines the conclusion that stunning currents below 100 mA AC cannot be recommended even with fast and efficient bleeding. Similar results can be observed for groups stunned with high frequencies of 800 and 1500 Hz, where all birds survived the stunning process independent of the amount of current (Figure 3). None of these stunning setups achieved an iso-electric EEG in more than 90% of the birds in both frequency bands, indicating an inadequate stun and a rapid recovery. Moreover, the increasing occurrence of corneal reflexes at 20 and 40 seconds post-stun and the high amount of spontaneous eye blinking support this judgement. Therefore, high frequency stunning from 800 Hz AC cannot be recommended with a stunning current of 150 mA or less.

Stunning setups inducing cardiac arrest

Frequencies with a maximum of 100 Hz at 100 mA and 200 Hz at 120 and 150 mA caused cardiac fibrillation in at least 80% of birds, thus preventing recovery. Both EEG frequency bands show a rapid decrease of birds without iso-electric EEG in P2 and P3, which is probably associated with brain death of the birds that did not recover. Electrocution has been discussed controversially. Gregory and Wotton (1987) recommend stun to kill methods inducing cardiac fibrillation, to avoid resumption of consciousness. This might on the other hand lead to carcass defects due to convulsions (Schütt-Abraham et al., 1983; Gregory, 1989). If cardiac arrest occurs in conscious birds, a painful experience cannot be excluded (Schütt-Abraham et al., 1983; Raj et al., 2006b). In the first 20 seconds post-stun, the EEG results in the 2-30 Hz band show that the percentage of birds lacking a profoundly suppressed EEG was higher than the percentage of birds that resumed breathing. This indicates that in the period directly following stunning total EEG power can be slightly elevated, although the birds will not recover from the stunning process. Moreover, the analogy of the result of the 2-30 Hz band and the absence of corneal reflexes, which indicate brain death, supports the assumption that this wide brain frequency band is more associated with overall brain function and therefore does not necessarily indicate perception and consciousness. A profound suppression of brain power in the 13-30 Hz band might therefore be more representative to indicate unconsciousness and absence of sensibility.

Successful stunning with limited effect on cardiac function

In the remaining groups, stunned with either 120 mA or 150 mA at 400 Hz and 100 mA at 200 Hz, the majority of animals resumed breathing and thus did not obtain cardiac arrest during stunning. Although the percentage of birds that showed a profoundly suppressed EEG in the 2-30 Hz band is below 90%, a rapid decrease can be observed (Figure 3). When the 13-30 Hz frequency band indicating sensitivity is considered, however, more than 90% of the birds show a profound EEG suppression up to 40 seconds post-stun. It could therefore be argued that these stunning setups are acceptable, though they do not cause cardiac fibrillation. The marked increase in the occurrence of corneal reflexes, however, may indicate a rapid recovery at 40 seconds post-stun. These electrical setups would therefore require a very quick and effective bleeding of chickens to avoid recovery before death from blood loss occurs.

Epileptiform activity and tonic-clonic convulsions

An initial occurrence of epileptiform activity before the profound suppression of the EEG has been interpreted to be indicative for stunning effectiveness in broiler chickens (Schütt-Abraham et al., 1983). Raj et al. (2006a) found a high incidence of epilepsy in broiler chickens stunned with alternating currents. A percentage of 90% epilepsy is reported for stunning setups with low frequencies and high currents (Raj et al., 2006a). Gregory and Wotton (1987) on the other hand found an attenuation of epileptiform activity with high currents. The results of the present study support these findings, as almost no epileptic seizure could be observed in the EEG of birds stunned with high currents and low frequencies. Due to the suppression of epileptic seizures with high stunning currents, the suitability of epilepsy as a measure to appraise stunning efficiency has been questioned (Gregory and Wotton, 1987). In the current study, epileptic activity could be found in a smaller percentage of birds as compared to previous studies (Raj et al. 2006a), and it occurs mainly in groups with higher stunning frequencies. This might be due to the long stunning time of 10 seconds. Gregory and Wotton (1987) found an average epilepsy duration of 17 and 12 seconds after the start of stunning. In a study on head-only stunning of broilers with different frequencies of an alternating current, Raj and O'Callaghan (2004a) found average epilepsy durations ranging from 9 seconds to 12 seconds, after a one second stunning time. It can therefore be assumed that in the present experiment, epilepsy might have occurred before the EEG recording started, due to the longer stunning time. Raj and O'Callaghan (2004a, b) found an increase in total brain power immediately after stunning, similar to the findings of the present study. This was interpreted to be caused by epileptic activity. Wormuth et al. (1981) identified epilepsy in all birds that experienced cardiac fibrillation in the waterbath, when current flow lasted for at least 4-6 seconds. Alternatively, birds might have passed directly into a phase of sudden neuronal death as suggested by Raj et al. (2006a). The occurrence of epilepsy is also supported by the outside appearance of the animals when leaving the waterbath. Birds stunned with high stunning currents showed very few tonic-clonic or clonic-only convulsions (Figure 5). Gregory (1989) described this phenomenon for birds that obtained cardiac arrest during stunning. Tonic-clonic seizures as described by Lee-Teng and Giaquinto (1969) mainly occurred with low stunning currents or high stunning frequencies in the present study (Figure 5). Schütt-Abraham et al. (1983) classified these as epileptic seizures. The consistency of the groups showing seizures in the EEG and tonic or clonic convulsions supports this view. It could be argued that the epileptiform fit occurred already in the waterbath or during transfer of the bird to the EEG clamp.

Evaluation of physical reflexes

The second aim of the present study was the evaluation of objective, physical parameters as a measure of consciousness in broiler chickens.

Breathing

As assumed in the previous discussion, resumption of breathing will occur in birds that did not develop cardiac arrest during stunning, and on its own is no indicator for returning consciousness (von Wenzlawowicz and von Holleben, 2001). With the resumption of breathing, however, oxygen supply to the brain will facilitate a full recovery.

Corneal reflex

In groups stunned with low frequencies and a minimum current of 100 mA, it can be observed that corneal reflexes can still occur in some birds initially that do not recover from the stunning process. The later corneal reflex test at 40 seconds post-stun shows a decreasing number of positive corneal reflexes in the same groups, where progressive brain death was identified in the EEG analysis. This is in line with findings of Gregory (1989), who describes the absence of the corneal reflex as an indication for approaching brain death or severe brain impairment, rather than just a loss of consciousness. It can therefore be assumed that absence of corneal reflexes in a considerable number of birds indicates deep unconsciousness or approaching death. The increase of corneal reflexes in many birds over time is a sign of progressive recovery. A positive response itself however does not necessarily mean that the bird is able to perceive pain. It is well known that the corneal reflex is a brain stem reflex and can be elicited in birds even under deep anaesthesia (Gregory, 1989). The consistency of the corneal reflex and the EEG results of the 2-30 Hz band indicates that this reflex can be a useful tool for commercial slaughter houses to evaluate deep unconsciousness or approaching death of the chickens. It can however be expected that a limited number of animals might still show a positive response for a short period post-stun. Under practical conditions a maximum of 30% of corneal reflexes is often used to identify acceptable stunning. The comparison of the EEG results with the occurrence of corneal reflexes in the present study (Figure 3) confirms this assumption.

Spontaneous eye blinking

Occurrence of spontaneous eye blinking was assessed as a physical reflex which might be more associated with consciousness of birds and thus a better measure to determine stunning efficiency in slaughter houses. The results presented in Figure 3 show that in those groups where the majority of animals recovered from stunning and EEG results did not indicate effective stunning, a high percentage of spontaneous eye blinks is observed. It can therefore be assumed that this behavioural reflex indicates recovery and signs of consciousness. In stunning groups where the majority of birds did not recover from stunning, only few birds showed spontaneous eye blinking. However, when stunned with 70 Hz and a minimum current of 100 mA, the percentage of birds with spontaneous eye blinking was higher than the percentage of birds that resumed breathing. As it is unlikely that birds recover without breathing for 40 seconds, occurrence of spontaneous eye blinks in these groups might indeed be explained with muscular fibrillation while the animal is passing away, rather than a sign of consciousness. This is supported by the fact that blinking occurred at a very high frequency and stopped abruptly after several seconds. Birds that recovered on the other hand showed regular periodic blinking that normally continued until the end of EEG recording. As it is difficult to distinguish the occurrence of spontaneous eye blinking as an expression of regaining consciousness from mere muscular vibration, a small number of birds with spontaneous eye blinks can be accepted in commercial slaughterhouses, while the animals can still be judged to be well stunned. A high increase of spontaneous eye blinks over time in combination with an increase in corneal reflexes indicates rapid recovery of animals. In an early stage post-stun not more than 15% of the birds should show spontaneous eye blinking, and at 30 seconds post-stun this can not be accepted in more than 30% of animals (Figure 3).

Wing flapping

Wing flapping did not prove to be indicative for consciousness in the present study. In contrast wing flapping seems to be more associated with convulsions after leaving the waterbath. This is obvious, as wing flapping occurred in most birds in those groups, where the majority of animals obtained cardiac arrest during stunning (Figures 3 and 4). If on the other hand wing flapping occurred during cardiac fibrillation, this could be an indication for severe convulsions of the breast muscle and therefore a sign for impaired carcass quality. From Figures 3 and 4 it is clear that at least 50% of the chickens in all stunning setups that achieved adequate stunning efficiency showed wing flapping. Further studies are required to better understand the implications of wing flapping and the application of different electrical parameters on carcass quality.

CONCLUSIONS

Electrical waterbath stunning using an alternating current proved to be effective to render broiler chickens unconscious. Stunning frequency has most impact on stunning efficiency and it appears that frequencies higher than 400 Hz cannot be recommended for a maximum stunning current of 150 mA. The amount of the stunning current has a similar importance on stunning efficiency and it is shown that stunning currents below 100 mA do not reach an adequate level of unconsciousness in birds. Broilers stunned with low frequencies and currents above 100 mA are not able to recover from stunning. Therefore an alternating current should be applied from 120 mA with a maximum of 400 Hz. If an AC current of 100 mA is used the maximum acceptable frequency is 200 Hz. The effect of these electrical setups on meat quality still has to be established. Rapid and effective bleeding is essential for setups where a frequency of 400 Hz is used to prevent the animals from regaining consciousness. The same applies for a stunning setup with 200 Hz with a stunning current of 100 mA. From the assessment of physical reflexes, the eye reflexes seem most reliable to evaluate unconsciousness. Both, corneal reflex and spontaneous eye blinking might still be visible in a limited number of birds, while an increase over time is a definite signal for recovery. A maximum percentage of 30% of positive corneal reflexes would therefore be acceptable, with a maximum of 15% of birds with spontaneous eye blinking at 20 seconds post-stun. Spontaneous eye blinking should never exceed 30%.

ACKNOWLEDGEMENT

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Chapter 5

Effects of waterbath stunning on the electroencephalograms and physical reflexes of broilers using a pulsed direct current

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ABSTRACT

Stunning efficiency of a pulsed direct current (DC) was assessed regarding the effect on the electroencephalogram (EEG) and physical reflexes. 467 broilers (males and females) were stunned in an electrified waterbath with 60, 80, 100, 120 and 150 mA at frequencies of 70, 100, 200, 400, 800 and 1500 Hz. Stunning time was 10 seconds. EEG recordings lasted for 120 seconds post-stun and simultaneously the occurrence of breathing, spontaneous eye blinking, corneal reflex and wing flapping was recorded. EEG records were assessed regarding a profound suppression to less than 10% of the pre-stun brain power in two brain frequency bands, 2-30 Hz and 13-30 Hz. The EEG results showed a significant effect of stunning frequency for all analysed parameters. Stunning frequencies of 800 and 1500 Hz did not achieve adequate stunning results. With a minimum stunning current of 120 mA at frequencies of 70 or 100 Hz or 150 mA at 200 Hz more than 80% of the animals did not resume breathing. Currents of 80 and 100 mA at 70 or 100 Hz achieved unconsciousness in more than 90% of the birds and birds recovered within 30-40 seconds post-stun. Epileptiform activity was found in a relatively low proportion of EEG traces. This could be explained by the long stunning time of 10 seconds, where epileptiform activity might have occurred already before the start of EEG recording. DC stunning causes less cardiac arrest during stunning, but occurrence of breathing seems more related to cardiac function than to consciousness. A maximum of 30% corneal reflexes and spontaneous eye blinking seems acceptable with a maximum of 15% spontaneous blinking at 15 seconds post-stun. 40% of wing flapping occurred in all effectively stunned groups. DC seems to have a different effect on male and female broilers as significantly more male broilers showed reflexes, while simultaneously the likelihood of profound EEG suppression was higher. Further investigation of this effect is necessary.

Keywords

broiler, stunning, pulsed direct current, electroencephalogram EEG, reflex

INTRODUCTION

A pulsed direct current is often used for electrical waterbath stunning in chicken slaughterhouses. Various combinations of currents, frequencies and stunning time are applied to optimise both, stunning efficiency and product quality. Under practical conditions frequencies between 50 and 200 Hz are often applied in European slaughterhouses with currents ranging from 60 o 150 mA. In recent scientific evaluations the effectiveness of a pulsed DC to render broiler chickens unconscious has been questioned (Raj et al., 2006b,c). However, the influence of the electrical parameters on the different tissues of broilers is still not well established and therefore assessment of stunning efficiency remains especially important.

Recording brain waves through the electroencephalogram (EEG) is the most objective available method to assess the state of (un)consciousness in broiler chickens. In EEG analysis, the occurrence of a profoundly suppressed iso-electric EEG as well as epileptiform activity is normally associated with loss of consciousness and therefore loss of sensibility (Schütt-Abraham et al., 1983; Raj et al. 2006a, b). Iso-electricity has been characterised by a reduction of the post-stun brain power to less than 10% of the pre-stun level (Raj and O'Callaghan, 2004a, b). Two EEG frequency bands have been considered regarding the occurrence of iso-electricity following stunning. Firstly the broader 2-30 Hz band represents all states of vigilance and consciousness and a profound suppression has been used to indicate loss of overall brain function (Raj and O'Callaghan, 2004a, b). The smaller band of 13-30 Hz represents information processing ability and a profound suppression has therefore been interpreted to indicate loss of sensitivity including pain (Raj and O'Callaghan, 2004a, b). The chicken EEG clamp (CHEC) has been developed as a non-invasive method to measure and assess EEGs of a large number of animals (Coenen et al., 2007). Using the CHEC, Prinz et al. (2009a) have analysed the typical spectral characteristics of a wake broiler chicken and thus, established the representative base-line EEG, which has been used to analyse reduction of brain power with different stunning setups.

Epileptiform activity on broiler EEG patterns as characterised with low frequency spike-wave discharges (Raj et al., 2006a) is considered as an indicator of unconsciousness. Moreover the occurrence of clonic-tonic or clonic-only convulsions in broilers leaving the waterbath has been associated with epileptiform activity (Schütt-Abraham et al., 1983).

In daily slaughterhouse practice assessment of behavioural reflexes following stunning is used to ensure good welfare standards (von Wenzlawowicz and von Holleben, 2001). The occurrence of breathing, spontaneous eye blinking and the response to corneal reflex test following waterbath stunning with a pulsed direct current has generally been assessed (Raj et al., 2006b), but a detailed systematic analysis of various combinations of stunning current and frequency is still not available.

The aim of the present study is twofold. Firstly, the effects of different amounts and frequencies of a pulsed direct current is analysed regarding their suitability to render broiler chickens unconscious in an electrical waterbath. This should take into consideration the range of stunning setups applied under practical conditions in European slaughterhouses. Secondly, the relationship between EEG analysis and behavioural reflexes is evaluated, to establish recommendations for welfare assessment in daily slaughterhouse practice.

MATERIAL AND METHODS

A total of 467 broiler chickens (Ross), 229 males and 238 females, were raised to seven weeks of age. The average weight was 1999 ± 211 g and 1668 ± 203 g respectively. The animals were individually stunned in an electrified waterbath, with their feet fixed into a grounded metal shackle which was attached to a rotating stand. The feet and shackle were sprayed with water to improve conductivity. Hanging upside down the birds' head was then immersed into the waterbath up to the base of the wings. The stunning bath consisted of a plastic basin with a metal plate as live electrode that covered the complete bottom. Salt was added to the water to maintain the conductivity at four millisiemens/cm. A commercially available stunning cabinet (Quest Cabinet, Meyn Food Processing Technology, Oostzaan, The Netherlands) delivered the pulsed direct current with a 50% duty cycle (pulse width 1:1). Frequencies of 70, 100, 200, 400, 800 and 1500 Hz were applied and for every frequency the voltage was adjusted to obtain the intended stunning currents of 60, 80, 100, 120 and 150 mA root mean square (rms). As the actual stunning current depends on the individual resistance of a bird, the amperage obtained was measured with an ampere meter. For the following analysis, birds were grouped according to the effectively obtained stunning current, thus leading to a small variance in group size. The number of animals that obtained a stunning current below 80 mA and above 150 mA was not sufficient to analyse these groups. The number of animals per group, average voltage and average current are given in Table 1.

Table 1. Number of animals, average current and voltage of the different stunning groups

	Stunning Frequency	**No. of animals**			**Average voltage**		**Average current**
	Hz	♂	♀	**Total**	♂ **(V)**	♀ **(V)**	**mA**
80 mA	70	6	4	**10**	69	91	88
	100	3	3	**6**	75	80	77
	200	6	6	**12**	72	95	80
	400	4	7	**11**	84	90	77
	800	6	10	**16**	82	97	84
	1500	9	5	**14**	83	98	83
100 mA	70	12	8	**20**	93	118	99
	100	6	5	**11**	102	125	100
	200	7	2	**9**	92	120	99
	400	10	8	**18**	91	117	98
	800	7	7	**14**	87	120	99
	1500	7	16	**23**	91	111	99
120 mA	70	5	9	**14**	105	133	119
	100	4	9	**13**	99	124	118
	200	7	11	**18**	102	135	120
	400	7	11	**18**	102	138	117
	800	13	5	**18**	105	125	119
	1500	10	9	**19**	119	142	120
150 mA	70	3	6	**9**	118	143	145
	100	10	11	**21**	111	149	144
	200	5	7	**12**	116	157	148
	400	11	6	**17**	124	165	143
	800	6	9	**15**	119	150	144
	1500	10	9	**19**	119	142	144
Total		174	183	**357**			

Average stunning time was 10.6 ± 0.7 seconds. Following stunning the rotating stand swung the birds towards the chicken EEG clamp (CHEC). The birds were fixed into the CHEC with the feet still hanging in the shackle. This procedure allowed EEG recording within 9.5 ± 4.3 seconds following stunning.

The EEG equipment and recording settings used in the present experiment have been described by Prinz et al. (2009b, unpublished). All recordings lasted for 120 seconds post-stun. Birds were subsequently euthanized in a box filled with carbon dioxide. During EEG recording the occurrence of spontaneous breathing, spontaneous eye blinking and wing flapping was observed and marked on observation channels on the EEG recordings. This facilitated a direct comparison of the birds' behaviour with changes in the brain waves. In addition the corneal reflex was tested every 20 seconds post-stun through touching of the birds' cornea with a feather. Neck tension was assessed 30 seconds after the animals left the waterbath. Since the heads were fixed into the CHEC, assessment of this parameter was considered not reliable and excluded from further analysis. Furthermore the state of the chickens' eyes (open or closed) was recorded immediately following stunning, as well as the occurrence of clonic-tonic convulsions. Convulsions were evaluated as described in Prinz et al. (2009b, unpublished).

EEG analysis was concentrated on the first 40 seconds post-stun, as this was judged to play a decisive role regarding animal welfare. An EEG analyzer (Brainvision Analyzer, Brain Products, 82205 Gilching, Germany, www.brainproducts.com), was used. EEG recordings were transferred to Brainvision Analyzer using a software-aid to convert Windaq-data (Dataq Instruments, Inc., Akron, OH. North America. www.dataq.com). To analyse total brain power, EEG recordings were filtered for 2-30 Hz and 13-30 Hz. The traces were divided into three post-stun periods, with P1 0-20 seconds, P2 20-30 seconds and P3 30-40 seconds post-stun. In every period three segments of one second were marked and a Fast Fourier Transformation calculated to obtain the total brain power in every segment. The grand average of the three segments provided the brain power of every post-stun period, which was expressed as a percentage of the representative wake chicken EEG, as described by Prinz et al. (2009a). This procedure allowed analysis of the EEG traces without artefacts caused by movements or manipulation of the birds when testing physical reflexes. For evaluation of the EEG records it was assessed, if animals obtained a profoundly suppressed or iso-electric EEG with less than 10% of a wake broiler chicken, considering both the 2-30 Hz band as well as the 13-30 Hz band. Birds that obtained an iso-electric EEG were considered to be unconscious, whereas failure of iso-electricity indicated inadequate stunning. In the visual evaluation of the EEG traces, spike-wave discharges with a frequency of 2-6 Hz were marked as typical characteristics for epileptiform seizures. A characteristic chaotic EEG pattern with high amplitude and low frequency directly after stunning, followed by an iso-electric EEG could be observed in many birds. This was also regarded as an indicator for a form of unconsciousness (Figure 1).

For the statistical analysis JMP 7.1 (2007) was used. Data was subjected to a Nominal Logistic Regression, where stunning current [mA], stunning frequency [Hz], the interaction of stunning current x stunning frequency and sex were fixed factors. The factor effects were calculated with the Chi-squared [χ^2] Likelihood Ratio Test. The Predicted Values were extracted to show the likelihood of birds not obtaining a profoundly suppressed EEG or showing physical reflexes.

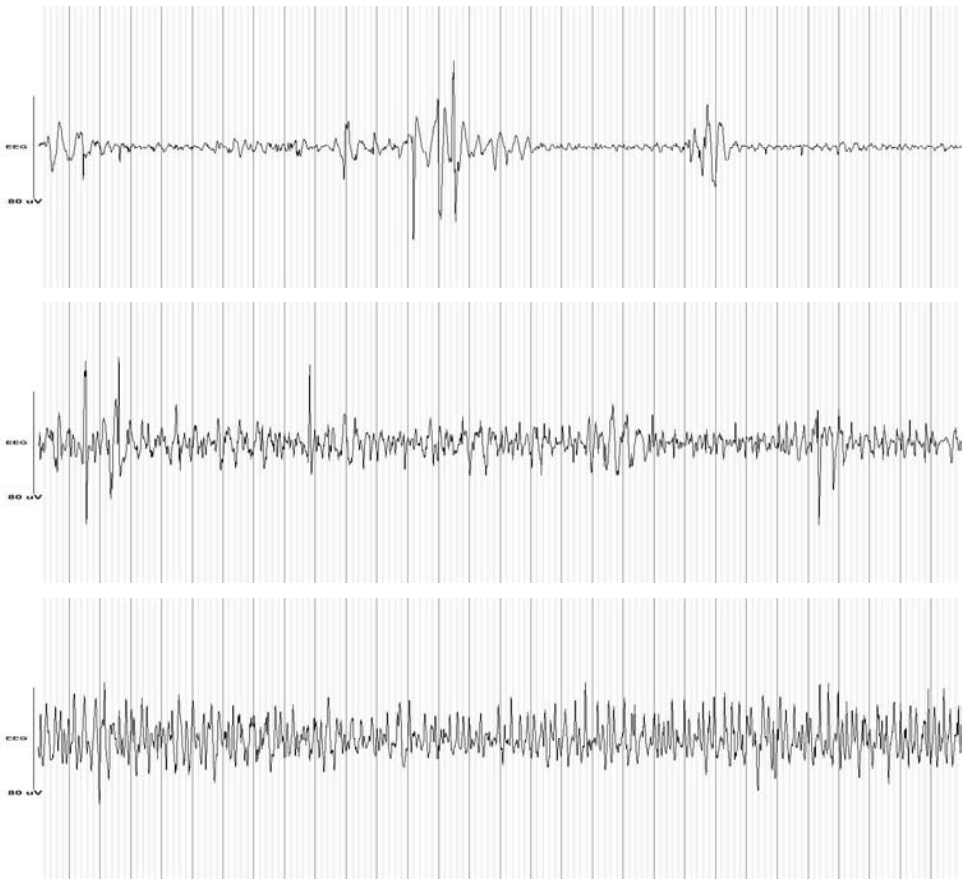


Figure 1. Examples of EEG traces of broiler chickens following waterbath stunning. The upper panel shows a profoundly suppressed, iso-electric EEG with some artefacts caused by the testing of physical reflexes. In the middle panel an EEG without a profound reduction of brain power is displayed. The lower panel contains an example of a chaotic EEG with high amplitude and low frequency. The amplitude of the Y-axis is 80 µV and the area between the bold vertical lines represent 1 second.

RESULTS

Fast Fourier Transformations were conducted for a total of 383 EEG traces, 197 for male and 186 for female broilers. Due to disturbances and movement artefacts 84 EEG records could not be analysed. For the evaluation of the EEG records in the first 40 seconds post-stun, the statistical analysis showed a strong significance for stunning frequency ($p<0.0001$) in both frequency bands. Stunning current was not significant, but the interaction of stunning frequency x current showed a significant effect in the broader band of 2-30 Hz ($p=0.0224$). Sex showed a significant effect in the 2-30 Hz band ($p=0.0038$), as well as in the 13-30 Hz band ($p=0.0155$). In both frequency bands, females were less likely to obtain a profoundly suppressed iso-electric EEG than males.

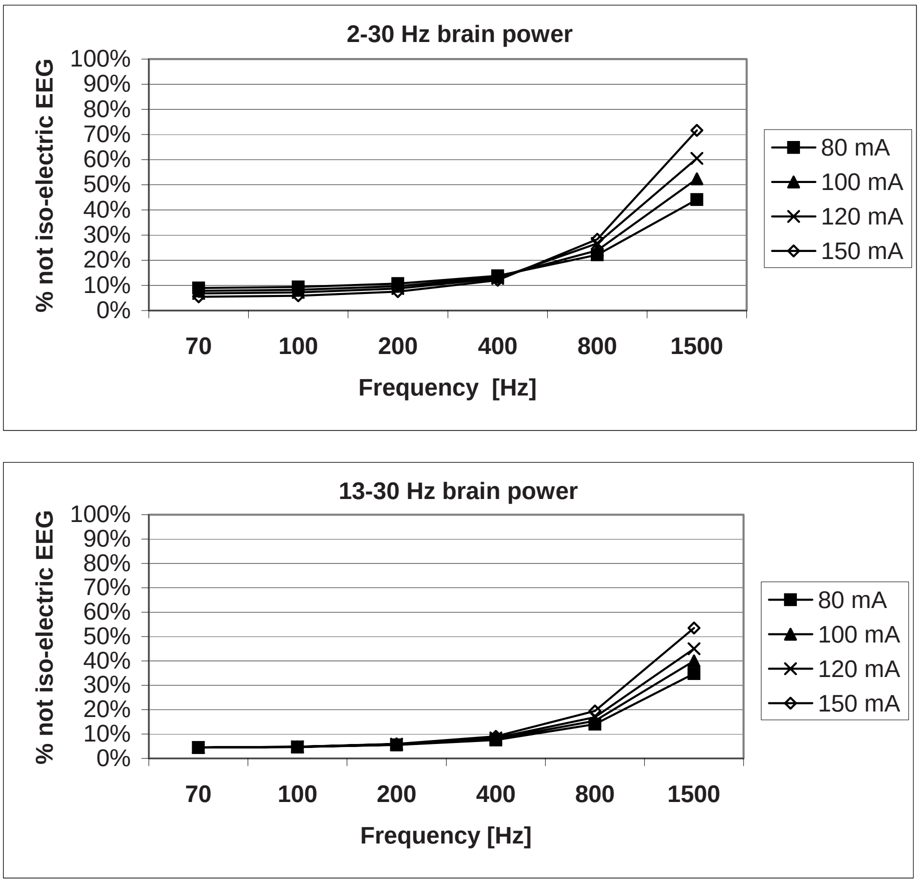


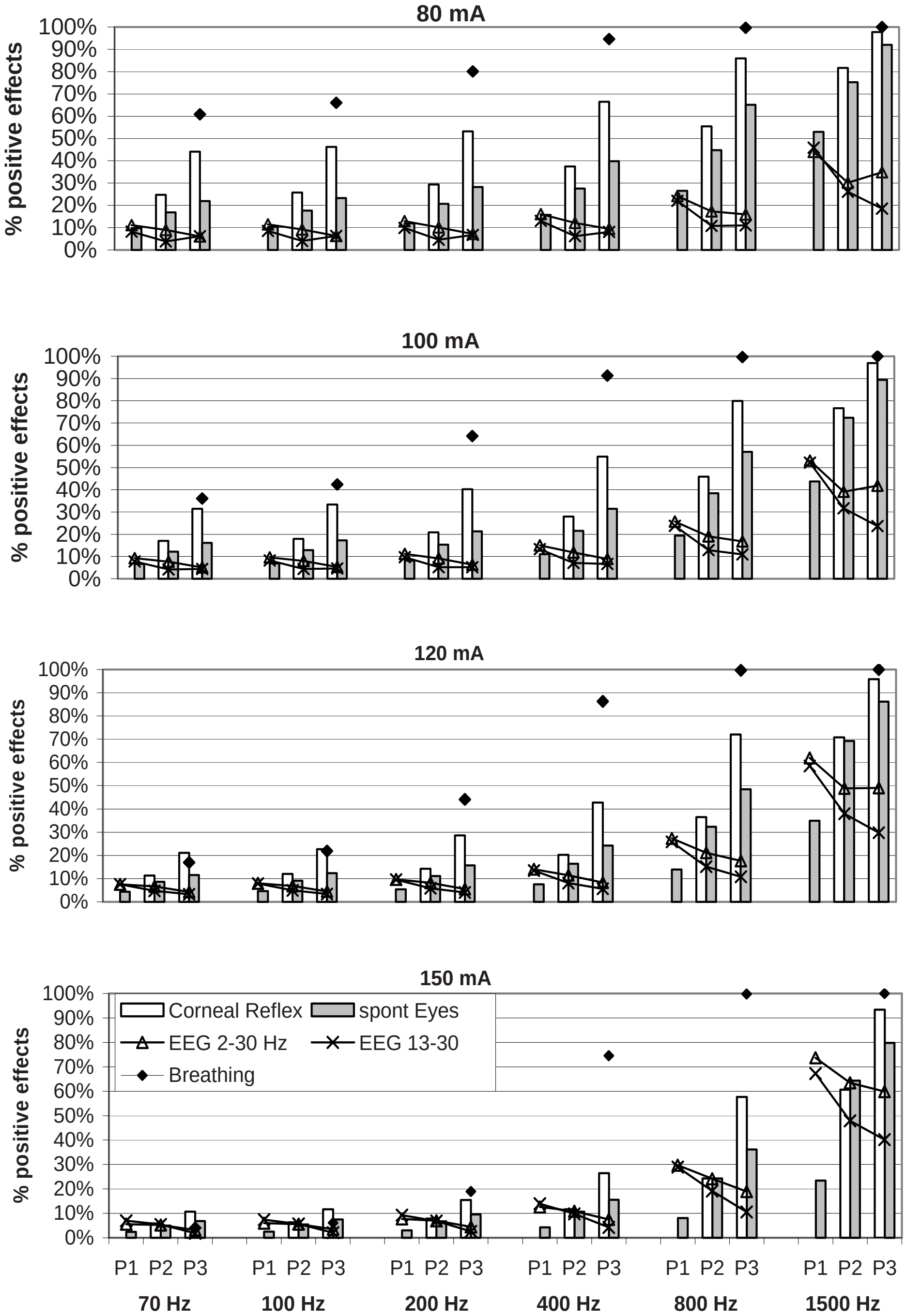
Figure 2. Percentage of birds not showing an iso-electric EEG pattern (less than 10% pre-stun power) in the first 40 seconds post-stun, for the broad brain frequency band of 2-30 Hz (upper panel) and the narrow frequency band of 13-30 Hz (lower panel).

Figure 2 shows the predicted values of the birds that did not obtain an iso-electric EEG in the first 40 seconds post-stun in the different electrical setups. Apparently for high frequencies (>400Hz) stunning efficiency was not adequate, with less than 90% of the birds showing a profoundly suppressed iso-electric EEG, independent of the stunning current. The results of the two brain frequency bands are similar, although the data of the 13-30 Hz band shows a slightly higher stunning efficiency than the 2-30 Hz band.

The predicted values for the birds not showing an iso-electric EEG in the three post-stun periods P1 (0-20 seconds), P2 (20-30 seconds) and P3 (30-40 seconds) are presented in Figure 3. Stunning frequency was significant in all three periods for both brain frequency bands 2-30 Hz and 13-30 Hz ($p<0.001$). High frequencies (>400 Hz) did not result in a high percentage of iso-electric EEGs in any of the post-stun periods. The percentage of iso-electricity was slightly lower in P1 in all stunning groups and increased in P2 and P3. Stunning current did not show significant effects for any post-stun period, but the interaction of stunning frequency and current showed significant effects in P2 and P3 for the 2-30 Hz band ($p=0.02176$ and $p=0.0422$ respectively), and in P3 also for the 13-30 Hz band ($p=0.0101$). Groups stunned with 150 mA showed a higher percentage of birds with an iso-electric EEG at 70 Hz than birds stunned with 80 mA at the same frequency. In P1, sex showed a significant effect in both frequency bands ($p=0.044$ and $p=0.0073$), and stunning efficiency was lower in females than in males.

The results of the eye reflexes showed some variation from the EEG. For the corneal reflex test at 20 and at 40 seconds post-stun both, stunning frequency and current were significant ($p<0.0001$), while the interaction of stunning frequency and current did not have a significant influence. Birds stunned with lower frequencies or higher currents were less likely to show corneal reflexes (Figure 3). The early corneal reflex test at 20 seconds post stun showed a significant effect for sex ($p=0.0077$) with more positive responses of males. At 40 seconds post-stun, sex effect was not significant. The interaction of stunning frequency and current did not have a significant influence on the corneal reflex test.

Occurrence of spontaneous eye blinking showed a significant effect for stunning current and stunning frequency in all three post-stun periods ($p<0.0001$) and for sex in P1 ($p=0.0006$), P2 ($p=0.0046$) and P3 ($p=0.0074$). In all three periods the probability of spontaneous eye blinking was lower for birds stunned with low stunning frequencies or high stunning currents. Males were more likely to show spontaneous eye blinking than females. The interaction of stunning frequency and current was not significant. The predicted values for positive responses to the corneal reflex test and the occurrence of spontaneous eye blinks are summarised in Figure 3.



The presence of breathing within the first 40 seconds post-stun indicated survival of chickens. For those birds that recovered from stunning, average time to resumption of breathing was 10.8 ± 3.9 seconds. Stunning frequency as well as the interaction of stunning frequency x current had a significant influence on the ability to recover ($p<0.0001$ and $p=0.0291$ respectively). In groups with a stunning frequency above 400 Hz all birds resumed breathing. When stunned with 150 mA at 70 Hz very few birds survived the stunning process, whereas application of 80 mA at the same frequency resulted in more than 50% of recovery. The effect of stunning current was not significant. The predicted values for the occurrence of breathing in the different stunning groups are summarised in Figure 3. Sex was of significant influence on the likelihood to resume breathing ($p=0.0005$), with a higher probability for the males as compared to female birds.

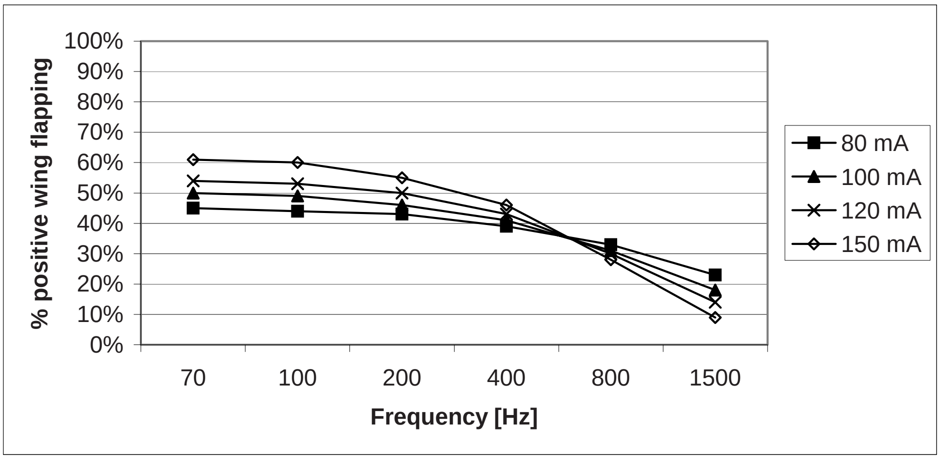


Figure 4. Percentage of wing flapping for different stunning setups

Vigorous wing flapping can lead to meat quality defects due to haemorrhages or broken bones. The effect of stunning frequency ($p<0.0001$) and interaction of stunning frequency x stunning current ($p=0.0067$) was significant for the occurrence of wing flapping while neither stunning current nor sex showed a significant effect. Wing flapping decreased with increasing frequency with the highest incidence for the setup of 150 mA at 70 Hz. Figure 4 shows the

Left Page: Figure 3. Percentage of birds not showing a iso-electric EEG (<10% pre-stun power) and spontaneous eye blinking in P1 (0-10 seconds), P2 (20-30 seconds) and P3 (30-40 seconds), corneal reflexes at 20 and 40 seconds post-stun and breathing in the first 40 seconds post-stun in response to stunning frequency and current.

probability of wing flapping in the different stunning groups extracted from the predicted values of the nominal logistic regression.

The occurrence of epileptiform activity was low in all stunning groups with increasing values in groups stunned with low currents and higher frequencies. The predicted values from the nominal logistic regression are presented in Figure 5. Both, stunning frequency and current showed a significant effect ($p<0.0001$), but no significant effect for the interaction of current x frequency or sex could be detected. Epileptiform activity was more likely in birds stunned with higher stunning frequencies, while higher stunning currents suppressed the occurrence of epileptiform activity. The presence of tonic-clonic or clonic-only convulsions showed a significant effect for stunning frequency ($p<0.0001$) and stunning current ($p=0.0203$). The likelihood for convulsions decreased with decreasing stunning frequency or increasing stunning current. The probability for convulsions in the different stunning groups is presented in Figure 5. Assessment of the animals' eyes when leaving the waterbath showed that most birds had their eyes open if the stunning frequency exceeded 100 Hz. The Effect Likelihood Test showed a significant effect for stunning frequency ($p<0.0001$), stunning current ($p=0.0419$) and sex ($p=0.02181$) with slightly more open eyes for male birds or low stunning current.

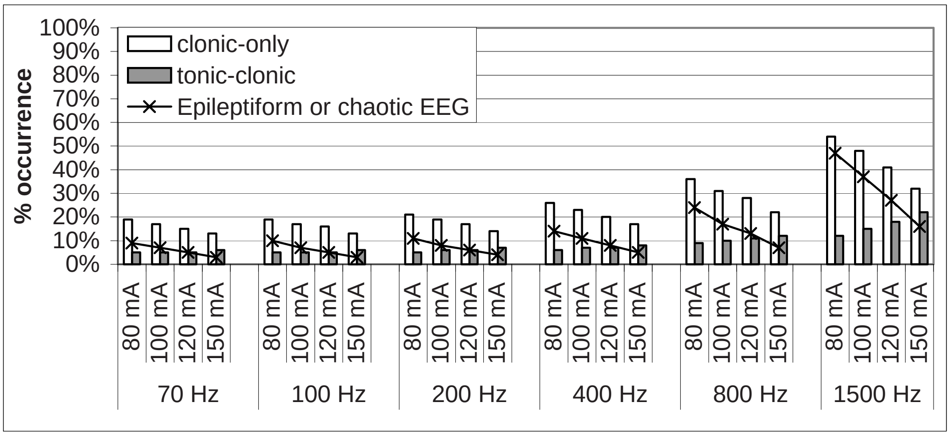


Figure 5. Percentage of animals showing an epileptiform or chaotic EEG and tonic-clonic or clonic-only convulsions after exiting the waterbath. Tonic-clonic convulsions are characterised by a rigidly backward bent neck and tucked wings accompanied by small and quick muscular contractions, followed by a relaxation of the body. In clonic-only convulsions vigorous wing flapping or large rhythmic contractions of the legs were observed.

DISCUSSION

The first aim of the present study was to determine electrical combinations that render a sufficient number of birds unconscious following electrical waterbath stunning with a pulsed direct current (DC). The range of electrical setups used in European slaughterhouses was considered to allow recommendations for daily practice.

Assessment of stunning efficiency using EEG analysis

A profoundly suppressed EEG to less than 10% of the pre-stun level in the 2-30 Hz band indicates loss of overall brain function, whereas the same profound suppression in the 13-30 Hz band has been used as an indicator for loss of sensibility including pain (Raj and O'Callaghan, 2004a, b). From Figure 2 it is obvious that stunning frequency has a main influence on stunning efficiency in the first 40 seconds post-stun. High frequencies above 200 Hz did not result in a profound suppression of the EEG in the 2-30 Hz band in at least 90% of the chickens independent of the amount of current. When an overall stunning efficiency of 85% of broilers is tolerated, stunning frequency can be increased to 400 Hz. However, more than 20% of the birds that were stunned with frequencies of 800 or 1500 Hz did not achieve a profound suppression of the EEG in the 2-30 Hz band, even with increasing stunning current. Frequencies above 400 Hz can therefore not be recommended with a maximum stunning current of 150 mA. The results of the 13-30 Hz band mainly support these findings with a slightly higher stunning effectiveness for all stunning frequencies and a deep suppression of the EEG in more than 90% of the broilers when stunned with 400 Hz, independent of the current. Considering the assumption that a profound suppression of the EEG in the 13-30 Hz band indicates loss of sensibility (Raj and O'Callaghan, 2004a, b) a pulsed direct current stunning with 400 Hz is acceptable. This corresponds with findings of Gregory and Wotton (1991) who concluded that a pulsed DC of 350 Hz and a minimum of 120 mA lead to an abolishment of somatosensory evoked responses in laying hens for 60 seconds post-stun. Loss of evoked potentials is generally interpreted as a disruption of primary pathways (Gregory and Wotton, 1989), indicative for loss of overall brain function. However, in the same study an influence of stunning current was found with ineffective stunning currents below 120 mA (Gregory and Wotton, 1991). This could not be confirmed in the present study.

EEG and behavioural parameters following stunning

Evaluation of the time till recovery of birds following electrical waterbath stunning is an important factor for animal welfare. The induction of ventricular fibrillation during stunning has welfare advantages, as recovery of the birds during bleeding is prevented (Gregory and Wotton, 1987). Moreover it allows more flexibility for the time of neck cutting (Gregory, 1998). On the other hand induction of cardiac arrest in potentially conscious animals could cause welfare concerns (Raj et al., 2006b). Moreover, possible carcass quality defects such as haemorrhaging and broken bones due to muscle convulsions have been reported (Gregory, 1989). However, ventricular fibrillation is not mandatory if recovery is prevented before death from bleeding supervenes (Raj, 2003).

The present study showed that the majority of birds survived the stunning process when stunned with frequencies higher than 200 Hz or currents below 100 mA. When stunned with at least 800 Hz all birds recovered from stunning. This corresponds with the findings of Prinz et al. (2009b unpublished), on broilers stunned with an alternating current (AC). In a study of Raj et al. (2006b) all broilers recovered from stunning with 800 or 1400 Hz pulsed DC. However, the present study clearly shows that unconsciousness is not achieved in a sufficient number of birds stunned with these high frequencies of 800 or 1500 Hz even when neck cutting is performed immediately (Figures 2 and 3).

Stunning setups inducing cardiac arrest

When stunned with low frequencies of 70 or 100 Hz and minimum stunning currents of 120 mA, at least 80% of the animals did not resume breathing and it can be assumed that they experienced cardiac arrest. The same could be observed for birds stunned with 150 mA and 200 Hz. These results correspond with the findings of Raj et al. (2006b), where 80% of the broilers showed cardiac fibrillation when stunned with 150 mA at 200 Hz. The results of the EEG in both frequency bands show a profound suppression in more than 90% of the broilers in the present study for all three post-stun periods (Figure 3). In addition, eye reflexes are absent in almost all birds directly following stunning and remain on a very low level until 40 seconds post-stun. As an iso-electric EEG in the 2-30 Hz band has been interpreted with loss of overall brain function (Raj and O'Callaghan, 2004a, b), it can be assumed that more than 90% of the animals are effectively stunned or even approaching brain death.

Successful stunning with limited effect on cardiac function

When stunning setups are sought that ensure an adequate period of unconsciousness and insensibility without inducing cardiac arrest, application of a pulsed DC proved to be effective. In the present study frequencies of 70, 100 and 200 Hz at currents of 80 or 100 mA resulted in a considerable number of birds that regained breathing following stunning (Figure

3). The profoundly suppressed EEG in both frequency bands for more than 90% of the birds indicate adequate stunning in these groups. This could not be observed for birds stunned with an alternating current of 80 mA with the same frequencies (Prinz et al., 2009b unpublished). Similar results have been obtained in the present study for birds stunned with 200 Hz at 120 mA and 400 Hz at 150 mA. The high increase of positive responses to the corneal reflex test together with rising spontaneous eye blinks, however, indicate the progressive recovery of the birds. Fast and efficient bleeding would therefore be necessary to prevent the recovery of animals, before death from bleeding supervenes. This is especially important for birds stunned with 80 mA, where death from bleeding must be ensured within 30 seconds post-stun.

Epileptiform activity and tonic-clonic convulsions

The occurrence of an epileptic fit followed by an iso-electric EEG has been interpreted as characteristic for adequate stunning, where this lasted for 30 seconds after the onset of current flow (Schütt-Abraham et al., 1983). Raj et al. (2006b) found a low occurrence of epileptiform activity after DC stunning and significantly less birds that were stunned with high stunning frequencies (800 or 1400 Hz) or lower stunning currents (100 mA) developed epileptiform activity . In the present study, epileptiform activity could only be observed on some EEG records (Figure 5). This corresponds with the findings of Prinz et al. (2009b unpublished) for birds stunned with the same amounts and frequencies of an alternating current. In comparison to the study of Raj et al. (2006b) epileptiform activity was considerably lower in the present study and increased with higher frequencies of 800 and 1500 Hz or lower currents of 80 and 100 mA (Figure 5). Gregory and Wotton (1987) also observed a suppression of epileptiform activity in birds stunned with high currents. The suitability of epileptiform activity as an indicator of stunning efficiency has therefore been questioned (Gregory and Wotton, 1987). The lower amount of epileptiform activity in the present study might be due to the difference in stunning time. While Raj et al. (2006b) stunned the animals for one second, the stunning time in the present study was 10 seconds. Raj et al. (2006a) reported average epileptiform activity duration from 9 seconds to 12 seconds after one second stunning with an alternating current. Gregory and Wotton (1987) found epileptiform activity lasting for 17 and 12 seconds after the onset of current flow. Considering the stunning time of 10 seconds, it can therefore be assumed that epileptiform activity occurred already in the waterbath or during transfer of the birds to EEG recording. Occurrence of clonic-tonic or clonic-only convulsions when leaving the waterbath support this conclusion. Schütt-Abraham et al. (1983) classified these phenomena as signs of epileptiform seizures, while a limb body would indicate cardiac arrest during stunning (Gregory, 1989). This corresponds with the results in the present study (Figure 3 and 5). Moreover, Wormuth et al. (1981) concluded that cardiac fibrillation is always associated with the occurrence of epileptiform activity, when current flow lasts for at least 4-6 seconds.

Evaluation of physical reflexes

The second aim of this study was to establish the suitability of physical reflexes as indicators for stunning efficiency under commercial conditions in a direct comparison of behavioural parameters with the EEG analysis.

Breathing

As previously discussed, the absence of breathing for up to 40 seconds post-stun was regarded as a sign of cardiac arrest. Resumption of breathing in recovering birds normally occurred within 20 seconds post-stun. It has been discussed that a pulsed direct current is less likely to induce cardiac arrest in chickens than AC (Kuenzel and Ingling, 1977). This could be confirmed in the present study, as the majority of birds only encountered cardiac arrest when stunned with frequencies of 70 and 100 Hz and minimum currents of 120 mA. In a similar study of Prinz et al. (2009b unpublished) on birds stunned with the same amounts and frequencies of AC, most birds encountered cardiac arrest when stunned with low frequencies and 100 mA or 200 Hz at 120 and 150 mA. In the present study, several stunning groups with a high proportion of breathing birds show a profound suppression of the EEG in all three post-stun periods, indicating an effective stun for at least 40 seconds post-stun. It can therefore be concluded that the occurrence of breathing is not directly related to consciousness, but to cardiac function. However, it must be considered that breathing will facilitate oxygen supply to the brain and thus facilitate recovery.

Corneal Reflex

Corneal reflex testing is a common procedure in commercial slaughterhouses to assess stunning efficiency. Wormuth et al. (1981) found that the corneal reflex remained absent in birds that developed cardiac fibrillation in the waterbath. Results of Raj et al. (2006c) correspond to these findings and the same can be observed in the present study (Figure 3). Moreover, groups with a profoundly suppressed EEG did not show a high proportion of corneal reflexes at 20 seconds post-stun (Figure 3). If birds regained breathing the incidence of corneal reflexes doubled at 40 seconds post-stun. However, a considerable proportion of corneal reflexes can be seen in groups with a profoundly suppressed EEG in both brain frequency bands. This supports the assumption that absence of the corneal reflex definitely indicates absence of sensibility, whereas positive responses are a sign of overall brain function, not necessarily related to consciousness. Gregory (1989) describes absence of the corneal reflex as indicator for approaching brain death or severe brain impairment, as it can still be elicited under deep anaesthesia. Von Wenzlawowicz and von Holleben (2001) characterised the return of corneal reflexes after stunning as a sign of the beginning recovery of brain functions. An increase of positive responses over time, therefore, shows the progressive recovery of a group of animals. The comparison of birds expressing a corneal reflex with the percentage of birds with a profoundly suppressed EEG in the present study (Figure 3) leads to the conclusion that a maximum of 30% of positive responses should not be exceeded under commercial conditions.

Spontaneous eye blinking

The occurrence of spontaneous eye reflexes has been analysed as a measure that might be more related to consciousness and sensibility, rather than mere brain function. Raj et al. (2006b, c) found a high consistency of birds with a positive corneal reflex and spontaneous blinking within 20 seconds post-stun. From the results presented in Figure 3 it is obvious that the percentage of spontaneous eye blinking is generally lower than the occurrence of corneal reflexes. Moreover, blinking remains absent in birds that do not recover from stunning. This is in line with results obtained by Raj et al. (2006c). On the other hand an increase of spontaneous blinking within the first 40 seconds can be seen in birds that resume breathing. A low amount of spontaneous eye blinking can even be observed in animals that show a deep suppression of the EEG in both frequency bands (Figure 3). It can therefore be assumed that an increase of corneal reflexes together with rising spontaneous eye blinks indicates a progressive recovery of the birds. For adequate stunning not more than 15% of eye blinking should be visible in a group of broilers at 20 seconds post-stun, and this value should not increase above 30% at 30 seconds post-stun.

Wing flapping

Assessment of wing flapping was included in the present study as an indicator for stunning effectiveness, but proved to be more related to convulsions following stunning. This is obvious as the majority of wing flapping occurred in those groups, where a high percentage of animals encountered cardiac arrest (Figure 3 and 4). Moreover, wing flapping mainly occurred in the first 40 seconds post-stun, but normally not in a later period in animals that recovered completely during EEG recording. However, wing flapping did not occur in all animals that encountered cardiac arrest. This might be important with regard to meat quality. Wing flapping might be an indicator for severe convulsions of the breast muscle, which could have a negative effect on product quality. From Figure 3 and 4 it is obvious that all groups with adequate stunning efficiency showed wing flapping in a range of 45% to 60%. Possible meat quality implications must, however, be assessed in a later study.

Gender effects

In the present study significant differences between male and female broilers regarding EEG results and physical reflexes have been observed. This is surprising, as voltage has been adjusted to obtain the same amount of current for all broilers in the same group. It could therefore be assumed that the resulting higher voltage used for female broilers caused the distinction (Table 1). This does not correspond with findings of Prinz et al. (2008b, unpublished) in a similar study on waterbath stunning using AC, where different voltage did not lead to significant differences in stunning results of male and female broilers. Moreover a significantly lower proportion of female birds obtained a profoundly suppressed EEG in the present study, while on the other hand female birds showed significantly less physical reflexes. This contradiction might be explained with the occurrence of epilepsy in females. Raj et al. (2004a, b) found an increase in overall brainpower in a period directly after stunning. They explain this high initial value with epileptiform activity. The significantly lower percentage of female birds obtaining an iso-electric EEG is limited to the P1 in the present study. Moreover, slightly more females showed epileptiform activity. This supports the assumption that the difference might be caused by epileptiform activity, which would explain the lower occurrence of physical reflexes. The reason for a possible higher incidence of epileptiform activity in female broilers, however, is not known, but a divergent effect of DC on females and males cannot be excluded. This is particularly important since under practical conditions male and female birds are normally stunned together.

CONCLUSION

Application of a pulsed direct current (DC) in a waterbath proves to be effective to render broiler chickens unconscious. Stunning frequency has a major effect on stunning efficiency, and frequencies exceeding 400 Hz do not produce adequate stunning results with a maximum of 150 mA. Frequencies of 70, 100 and 200 Hz with minimum currents of 120 mA cause cardiac arrest in the majority of birds, thus preventing recovery of animals post-stun. Lower currents of 80 and 100 mA applied with the same frequencies do not show this effect on cardiac function, but still prove to render the animals unconscious for an adequate period post-stun. A frequency of 400 Hz is only effective when applied with 150 mA. In all groups where a high percentage of birds resumed breathing, an increase of corneal reflexes and spontaneous eye blinking after stunning indicates the progressive recovery of animals. Rapid and efficient bleeding is therefore mandatory with these stunning setups to prevent recovery of the animals. This is especially important for stunning setups of 80 mA, where death from bleeding must be ensured within 30 seconds post-stun. Eye reflexes seem most reliable to evaluate unconsciousness. Both, corneal reflex and spontaneous eye blinking might still be visible in a limited number of birds, while an increase over time is a definite signal for recovery. A maximum percentage of 30% of positive corneal reflexes would therefore be acceptable, with a maximum of 15% of birds with spontaneous eye blinking at 20 seconds post-stun. Spontaneous eye blinking should never exceed 30%.

ACKNOWLEDGEMENT

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Chapter 6

Electrical waterbath stunning: influence of different waveform and voltage settings on the induction of unconsciousness in male and female broiler chickens

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ABSTRACT

Stunning effectiveness of male and female broiler chickens was analysed in response to different waveforms at three constant voltage levels. 180 male and female broiler chickens were stunned using a sine wave alternating current (AC) of 50 Hz, rectangular AC of 70 Hz and pulsed direct current (DC) of 70 Hz with a constant voltage of 60, 80 and 120 V. In each stunning group 10 male and 10 female birds were stunned for four seconds. The current obtained by every bird was recorded. For stunning efficiency the electroencephalogram (EEG) and physical reflexes were analysed. The EEG was recorded for 120 seconds post-stun. Simultaneously the occurrence of spontaneous eye blinking, wing flapping and breathing was assessed and the corneal reflex was tested every 20 seconds post-stun. The EEG was analysed regarding the occurrence of a profound suppression to less than 10% o the pre-stun level in the 2-30 Hz band and 13-30 Hz. Female broilers showed a higher resistance and consequently obtained a significantly lower stunning current. This resulted in a lower stunning efficiency for females, when the same constant voltage was applied to males and females. The waveforms required different amounts of currents to achieve 90% stunning efficiency. A minimum of 70, 90 and 130 mA could be established for sine wave AC, rectangular AC and pulsed DC respectively. The low stunning efficiency of pulsed DC might be caused by the short stunning time of four seconds. This effect should be further investigated for DC stunning, while no effect for AC could be detected. Very few birds stunned with AC resumed breathing following stunning, indicating cardiac arrest. Pulsed DC showed a lower effect on cardiac function. The level of wing flapping, indicating convulsions and possible meat quality defects was higher for the AC treatments.

Keywords

broiler, stunning, alternating current, pulsed direct current, electroencephalogram

INTRODUCTION

Electrical waterbath stunning of broiler chickens is the standard method in commercial slaughterhouses to render the animals unconscious before slaughter. Various combinations of electrical waveforms as well as frequency and amount of current are used with different effects on animal welfare and meat quality. A sinusoidal alternating current (AC) of 50 Hz with a minimum of 120 mA has been reported to induce cardiac arrest in 100% of the broilers (Wormuth et al., 1981). In recent years different waveforms have been introduced such as square wave alternating currents or pulsed direct currents (DC) with high frequencies. However, the effect of the different electrical parameters on the induction of unconsciousness is not fully understood (Raj and Tserveni-Gousi, 2000).

Assessment of brain waves through recording of electroencephalograms (EEG) is the most reliable method to understand the state of (un)consciousness following stunning. The “chicken EEG clamp – CHEC” (Coenen et al. 2007) is a non-invasive method, which allows derivation of broiler brain waves and assessment of different electrical setups on a great number of birds.
The occurrence of a flat, iso-electric EEG with a profound reduction of electrical brainpower to less than 10% of the pre-stun level has been used to indicate unconsciousness in broilers (Raj et al. 2006a,b). Two brain frequency bands have been considered for assessment of iso-electricity. A profound reduction in the 2-30 Hz band has been associated with an overall loss of brain function (Raj and O’Callaghan, 2004a, b). The smaller band of 13-30 Hz has been associated with information processing ability or the ability to perceive pain. A profound reduction of the 13-30 Hz band has therefore been used as an indicator for insensibility in broilers (Raj and O’Callaghan, 2004a, b). Prinz et al. (2009a) analysed the brainpower of wake broiler chickens in these two frequency bands using the CHEC and thus established a representative base-line EEG. This base-line EEG can be used to calculate the reduction of electrical brainpower following different stunning setups. Epileptic activity prior to the iso-electric EEG has also been used as an indicator for adequate stunning efficiency (Schütt-Abraham et al., 1983; Raj et al., 2006a,b).

In addition to EEG analysis, physical reflexes are often used to assess stunning effectiveness. Prinz et al. (2009b,c unpublished) analysed the occurrence of different reflexes following waterbath stunning with AC and DC currents of various frequencies. In their studies, positive responses to the corneal reflex test and occurrence of spontaneous eye blinking were most reliable to indicate returning consciousness. More than 30% of eye reflexes and a marked increase of positive responses over time have been interpreted with regaining consciousness (Prinz et al., 2009b,c unpublished). Resumption of breathing was not directly related to consciousness but indicated failure to induce cardiac arrest during stunning (Prinz et al., 2009b,c, unpublished). Induction of cardiac arrest in the waterbath prevents recovery of the birds during bleeding (von Wenzlawowicz and von Holleben, 2001). Due to muscle convulsions it might be associated with meat quality defects (Gregory, 1989).

The aim of the present study is to understand the effects of three different waveforms (sine wave AC, rectangular AC and pulsed DC), on the induction of unconsciousness and death in both male and female broilers. To identify the condition of the animals directly following stunning, the EEG was measured. Additionally a number of physical reflexes such as breathing, eye blinking, and wing flapping together with the corneal reflex were assessed.

MATERIAL AND METHODS

A total of 180 Ross broiler chickens, 90 males and 90 females, were raised in one flock for 7 weeks. The final weight was 2.6 ± 0.2 and 2.3 ± 0.2 for males and females respectively. Birds were then randomly allocated to three experimental groups. Three different waveforms sine wave AC, rectangular AC and pulsed DC were tested with three constant voltage settings. All experimental groups consisted of 10 male and 10 female broilers. For stunning, single birds were immersed into an electrified waterbath up to the base of their wings. The animals' feet were fixed into a grounded metal shackle, which was mounted onto a rotating stand. Before stunning the feet and shackle of every bird were sprayed with water to reduce the electrical resistance. The waterbath consisted of a plastic basin equipped with a metal plate as live electrode covering the complete bottom. Salt was added to the water to keep the conductivity constant at four Millisiemens/cm. A constant voltage of 60, 80 and 120 Volts was delivered from an electrical stunning cabinet (Quest Cabinet, Meyn Food Processing Technology, Oostzaan, The Netherlands). The r.m.s. current (root mean square) per bird was determined by the individual resistance of each broiler and was measured using a scope and current probe (123 Industrial Scope Meter {20 MHz}and 80i-110s AC/DC, Fluke Corp., Everett, USA). All data was recorded on to a data acquisition programme (View SW90W, Fluke Corp.). In all stunning groups a low frequency was used. For technical reasons sine wave AC was applied with 50 Hz and rectangular AC and pulsed DC with 70 Hz. The pulse width in the DC treatments was 1:1 (50% duty cycle). Average stunning time was 4.2 ±0.8 seconds in all groups.

Following stunning the chickens were fixed into the EEG device while still hanging in the shackle. Brain waves were recorded within 10 seconds post-stun. The EEG equipment and recording settings used in the present experiment have been described by Prinz et al. (2009b, unpublished). All EEG recordings lasted for 120 seconds post-stun and birds were subsequently euthanized in a box filled with carbon dioxide. During EEG recording the occurrence of breathing, spontaneous eye blinking and wing flapping was recorded on observation channels on the EEG, thus facilitating a direct comparison between brain state and physical reflexes. Failure of resumption of breathing following stunning was regarded as a sign of cardiac arrest. The corneal reflex was tested through touching of the birds' cornea with a feather every 20 seconds post-stun. Neck tension was tested 30 seconds post-stun, but due to the fixation of the broilers in the CHEC, assessment was difficult and this parameter was excluded from analysis. Directly following stunning the occurrence of tonic or clonic convulsions was assessed and recorded as described by Prinz et al. (2009b, unpublished).

All EEG recordings were transferred to an EEG analyzer (Brainvision Analyzer, Brain Products, 82205 Gilching, Germany, www.brainproducts.com) using a Software-aid to convert Windaq-data (Dataq Instruments, Inc., Akron, OH. USA. www.dataq.com). The recordings were filtered for the broader frequency band of 2-30 Hz and for the smaller band of 13-30 Hz. They were then subdivided into three post-stun periods: P1 0-20 seconds, P2 20-30 seconds and P3 30-40 seconds. In each period five segments of one second were marked and a Fast Fourier Transformation (FFT) calculated, showing the total brainpower content of every segment. The Grand Average FFT of the five segments in each period delivered the representative brainpower in the respective period. This facilitates a comparative analysis of genuine EEGs without the influence of disturbances caused by movement artefacts or manipulation of the animal during testing of physical reflexes. The brainpower thus obtained from the three post-stun periods was expressed as a percentage of the representative base-line EEG (Prinz et al., 2009a). The relative brainpower was used to evaluate the level of (un)consciousness in the broilers. A reduction in total brainpower to less than 10% of the base-line EEG in the 2-30 Hz was regarded as a profoundly suppressed or iso-electric EEG with loss of overall brain function. The same profound reduction in the 13-30 Hz band was considered to be indicative for loss of sensibility (Raj and O'Callaghan, 2004a,b). Relative brainpower of more than 10% of the base-line EEG indicated inadequate stunning. In a visual assessment of the recordings, epileptic activity was marked where the EEG showed typical spike and wave discharges with a frequency of 2-6 Hz (Figure 1). A characteristic chaotic EEG pattern with high amplitude and low frequency directly after stunning, followed by an iso-electric EEG could be observed in many birds. This was also regarded as an indicator for a form of unconsciousness

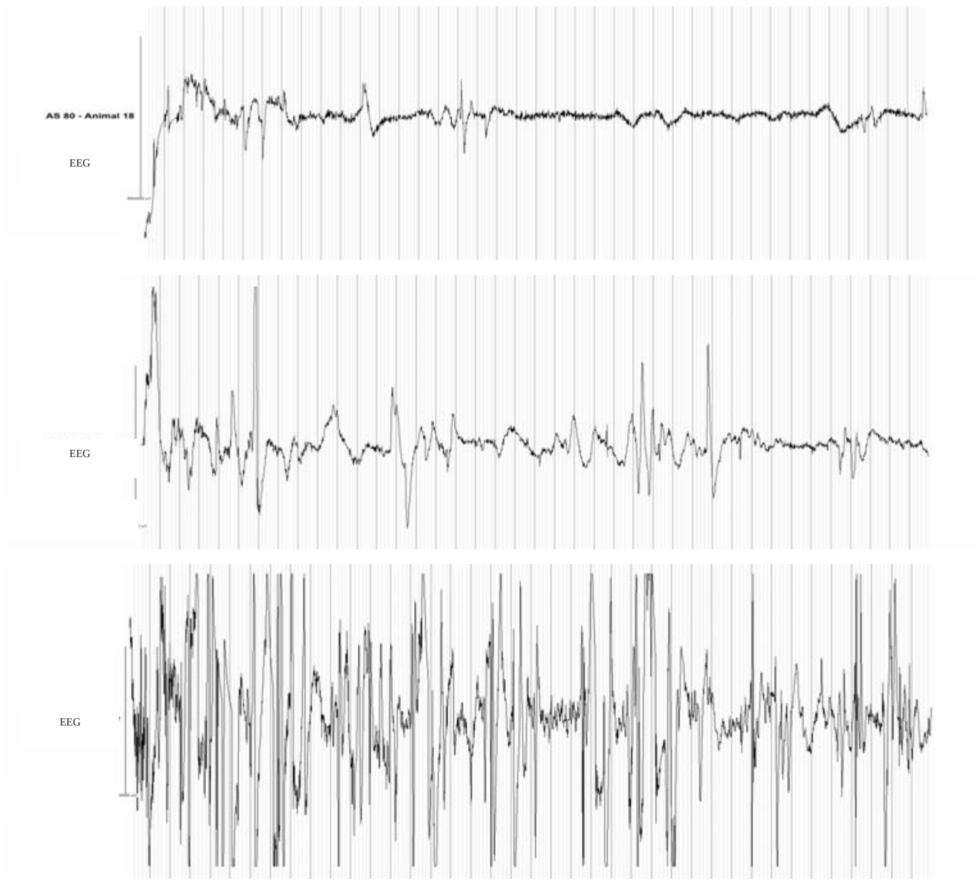


Figure 1. Examples of EEG traces of broiler chickens following waterbath stunning with different waveforms: sine wave AC in the upper panel, rectangular AC in the middle panel and pulsed DC in the lower panel. The amplitude of the Y-axis is 80 µV and the area between the bold vertical lines represent 1 second.

For statistical analysis JMP 7.1 (2007) was used. The data was submitted to Nominal Logistic Regression. All factor effects were calculated with the Chi-squared [χ^2] Likelihood Ratio Test with waveform, voltage [V], sex and the interactions of waveform x sex and waveform x voltage as fixed factors. From the predicted values the likely percentage of birds not showing a profoundly suppressed EEG or expressing positive physical reflexes was obtained and plotted.

RESULTS

Analysis of the current per bird showed a significant effect for sex and waveform. The interaction of waveform x sex was not significant (Table 2). With the same constant voltage setting, female birds obtained a significantly lower current than males in all stunning groups. The same voltage setting achieved a significantly higher current with sine wave AC compared to rectangular AC. Application of a pulsed DC resulted in the lowest current per bird (Table 1).

Table 1. Means and SD of the current obtained by male and female broilers in response to different waveforms.

	Voltage [V]	Current per broiler [mA]	
		Males	Females
Sine wave AC	60	72 ± 8	52 ± 10
	80	92 ±14	73 ±6
	120	174 ± 22	138 ± 15
Rectangular AC	60	67 ± 6	48 ± 7
	80	91 ± 12	71 ± 9
	120	153 ± 21	117 ± 15
Pulsed DC	60	61 ± 7	36 ± 10
	80	78 ± 17	57 ± 10
	120	128 ± 24	103 ± 15

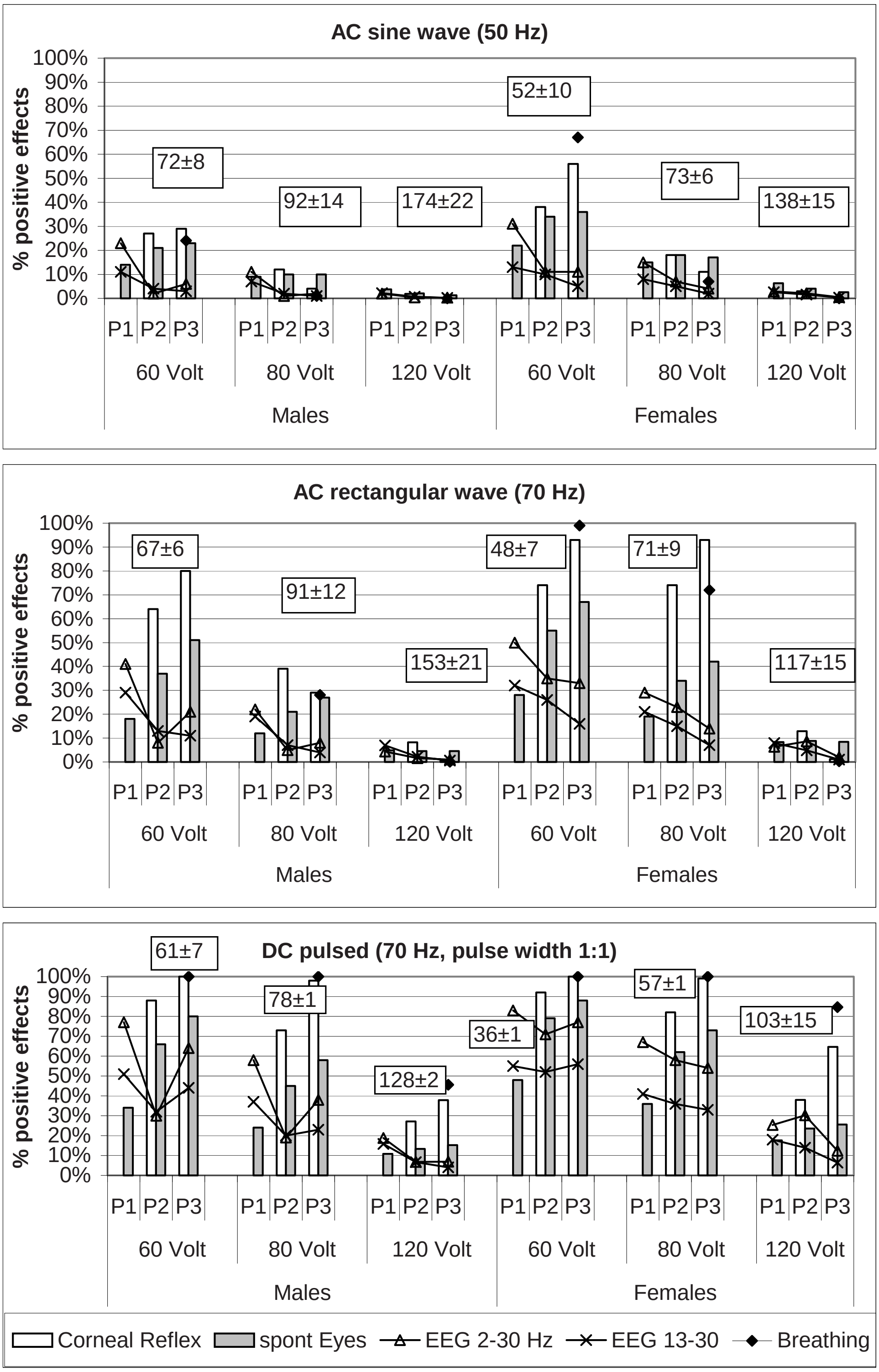
FFTs were calculated for a total of 164 EEG recordings, while 16 EEG traces (10 for males and 6 for females) could not be analysed due to movement artefacts and disturbances. The occurrence of an iso-electric EEG in the 2-30 Hz band EEG in the first 40 seconds post stun was significantly influenced by waveform, voltage and sex (Table 2). Increasing voltage resulted in more birds showing a profoundly suppressed EEG in this frequency band (Figure 2). When stunned with sine wave AC the occurrence of an iso-electric EEG was more likely than with a rectangular AC, while DC showed the lowest stunning effect (Figure 2). Female birds showed significantly less profoundly suppressed EEGs than male broilers (Figure 2).

Analysis of the 13-30 Hz band showed similar results with significant effects of waveform and voltage, while sex was close to the level of significance (Table 2, Figure 2).

Analysis of the EEG recordings and physical reflexes in the three post-stun periods P1, P2 and P3 allow a better understanding of the stunning effect in the different groups. In P1 a significant effect of voltage was detected in the 2-30 Hz band (Table 2). In the smaller frequency band of 13-30 Hz no factor showed a significant effect and the interaction of waveform x sex was close to the level of significance (Table 2). With increasing voltage more animals showed a profoundly suppressed EEG in both frequency bands (Figure 2). In P2 the effect of voltage, sex, waveform and the interaction of waveform x sex in the 2-30 Hz band were significant (Table 2). In the 13-30 Hz band voltage and waveform showed a significant effect while sex was close to the level of significance (Table 2). In P3 again stunning voltage, sex and waveform showed a significant effect in the 2-30 Hz band, but not the interaction of the factors (Table 2). In the 13-30 Hz band again voltage and waveform significantly influenced the occurrence of iso-electric EEGs in the broilers (Table 2). In both frequency bands more animals showed an iso-electric EEG with higher stunning voltage. Males were more likely to obtain an iso-electric EEG than females. Sine wave AC was most effective followed by a rectangular AC and pulsed DC (Figure 2).

Analysis of the physical reflexes showed a significant effect of all factors on the occurrence of the corneal reflex at 20 seconds post-stun (Table 2). At 40 seconds post-stun only the interaction of waveform x voltage did not show a significant influence on the corneal reflex (Table 2). Birds were more likely to express a positive response when stunned with lower voltage or pulsed DC, while sine wave AC stunning resulted in the lowest percentage of corneal reflexes. Females showed more positive reflexes than males. The occurrence of spontaneous eye blinking was significant for all factors in all three post-stun periods (Table 2). Animals stunned with higher voltage expressed less spontaneous eye blinking, Sine wave AC suppressed the occurrence of this reflex, compared to rectangular AC (Figure 2). Birds stunned with pulsed DC showed most spontaneous eye blinking. Female expressed more spontaneous eye blinking than males in all groups (Figure 2).

Right Page: Figure 2. Percentage of birds not showing an iso-electric EEG (<10% pre-stun power) and percentage of birds with positive reflexes: corneal reflex, breathing and spontaneous eye blinking in different periods post-stun: P1 0-20 seconds, P2 20-30 seconds, P3 30-40 seconds. Numbers in the boxes show the average current in the groups and standard deviation.


AC sine wave (50 Hz)
% positive effects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
72±8
92±14
174±22
52±10
73±6
138±15
P1 P2 P3 P1 P2 P3 P1 P2 P3 P1 P2 P3 P1 P2 P3 P1 P2 P3
60 Volt 80 Volt 120 Volt 60 Volt 80 Volt 120 Volt
Males
Females
AC rectangular wave (70 Hz)
67±6
91±12
153±21
48±7
71±9
117±15
DC pulsed (70 Hz, pulse width 1:1)
61±7
78±1
128±2
36±1
57±1
103±15
Corneal Reflex
spont Eyes
EEG 2-30 Hz
EEG 13-30
Breathing

Failure to resumption of breathing indicated the occurrence of cardiac arrest in the different stunning groups. Stunning voltage and waveform, sex and the interaction of waveform x sex proved to be significant. Broilers were less likely to resume breathing when stunned with higher voltage or sine wave AC as compared to lower voltages or pulsed DC (Figure 2). Significantly more males obtained cardiac arrest (Figure 2).

The results for the occurrence of wing flapping showed an opposite development with significantly more birds expressing wing flapping when stunned with sine wave AC compared to rectangular AC or pulsed DC (Figure 3). The effect of voltage was close to the level of significance. Higher voltage settings produced more wing flapping (Figure 3). Female broilers were significantly more likely to flap their wings than males (Figure 3). The interaction of waveform x voltage also showed a significant effect. Increasing voltage with the same waveform resulted in more birds with severe wing flapping.

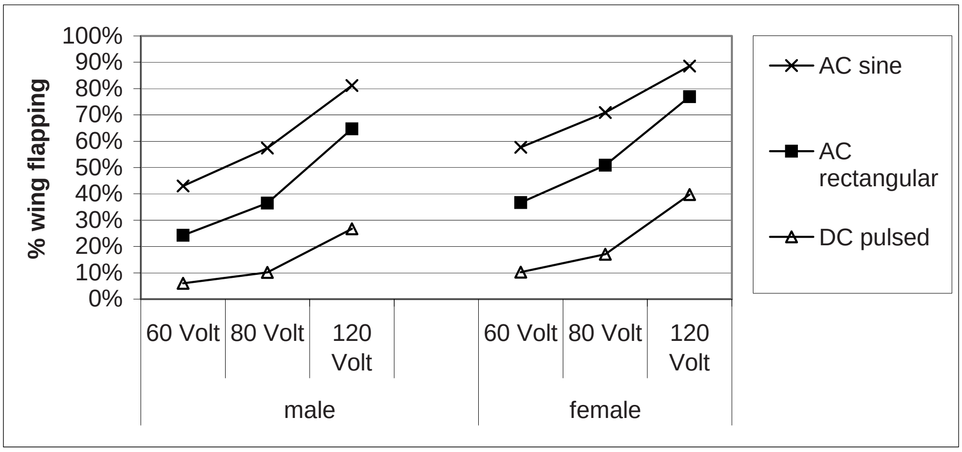


Figure 3. Percentage of wing flapping in the different stunning setups

The occurrence of epileptic activity was significantly influenced by all factors and was generally low in all stunning groups (Table 2, Figure 4). Birds stunned with sine wave AC were less likely to express epileptic activity than animals stunned with rectangular AC or pulsed DC. Increasing voltage suppressed the occurrence of epileptic activity. Females generally showed more epileptic activity than males (Figure 4). When leaving the waterbath a high amount of birds showed tonic-clonic convulsions (81 to 100%, means not shown).

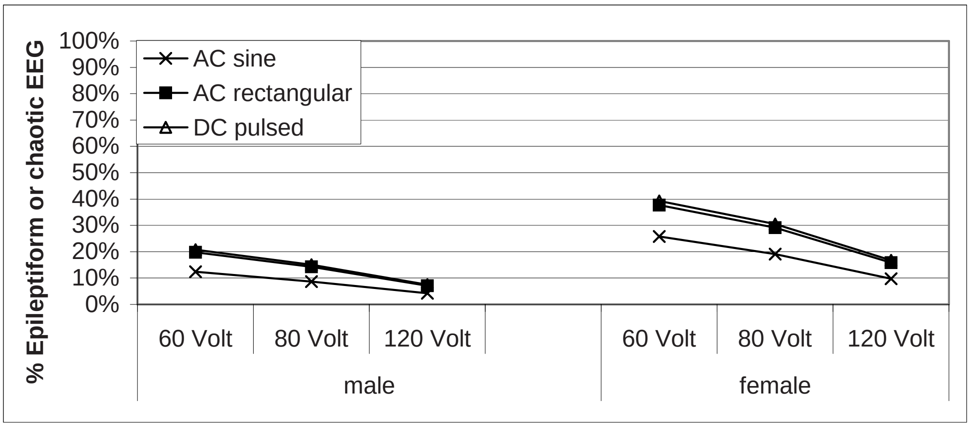


Figure 4. Occurrence of an epileptiform or chaotic EEG (low frequency epilepsy and slow wave disturbance) in the different stunning groups

Table 2. Results of the statistical analysis: Nominal Logistic Regression

Factor Effects	**Voltage**	**Waveform**	**Sex**	**Waveform x Voltage**	**Waveform x sex**
Current		p=0.0077	p<0.0001		n.s.
EEG 2-30 Hz (P1-P3)	p<0.0001	p<0.0001	p=0.0138	n.s.	n.s.
EEG 13-30 Hz (P1-P3)	p=0.0006	p=0.0004	*p=0.0694*	n.s.	n.s.
EEG P1 2-30 Hz	p=0.0038	n.s.	n.s.	n.s.	n.s.
EEG P1 13-30 Hz	n.s.	n.s.	n.s.	n.s.	*p=0.0596*
EEG P2 2-30 Hz	p=0.004	p=0.0177	p=0.0077	n.s.	p=0.0408
EEG P2 13-30 Hz	p=0.0035	p=0.0052	p=0.0953	n.s.	n.s.
EEG P3 2-30 Hz	p=0.0002	p<0.0001	p=0.0487	n.s.	n.s.
EEG P3 13-30 Hz	p=0.0008	p<0.0001	n.s.	n.s.	n.s.
Corneal Reflex 20 sec	p<0.0001	p<0.0001	p=0.0113	p<0.0001	p<0.0001
Corneal Reflex 40 sec	p<0.0001	p<0.0001	p=0.0041	n.s.	p=0.0004
Spont. Eyes P1	p<0.0001	p<0.0001	p=0.0127	p<0.0001	p=0.0051
Spont. Eyes P2	p<0.0001	p<0.0001	p=0.0038	p=0.0004	p=0.0008
Spont, Eyes P3	p<0.0001	p<0.0001	p=0.018	p=0.0003	p=0.0002
Breathing	p<0.0001	p<0.0001	p=0.0001	n.s.	p=0.0013
Wing flapping	*p=0.0663*	p<0.0001	p=0.0249	p<0.0001	n.s.
Epileptic activity	p=0.0008	P=0.0048	p=0.0011	p=0.0013	p=0.008
Clonic-tonic convulsions	p<0.0001	p<0.0001	p=0.0004	n.s.	n.s.

DISCUSSION

The aim of the study was to determine the different effect of three waveforms on stunning efficiency and induction of cardiac arrest considering three different voltage settings.

Electrical resistance

Firstly it is very obvious that the same constant voltage resulted in a significantly different electrical current delivered to the birds, depending on waveform and sex. Birds stunned with voltage of a sine wave AC resulted in higher amperage per bird than application of a rectangular AC voltage. Birds stunned with pulsed DC of the same voltage obtained the lowest current (Table 2). Bilgili (1992) reports that higher voltage was necessary with pulsed DC to obtain the same amperage when compared to AC in a pilot processing plant.

Female broilers obtained a significantly lower current in all stunning groups than male birds treated with the same constant voltage (Table 2). The lower body weight of female birds did not cause the distinction as this factor was corrected in the statistical analysis. Wormuth et al. (1981) did not find a different resistance between male and female broilers in their study, while laying hens showed a significantly higher resistance. Rawles et al. (1995) report a significantly higher voltage use for females to obtain the same current as compared to males. Corresponding to the results presented here, body weight could be excluded as the cause for the distinction in their study. However, a physiological difference with more highly resistant abdominal fat in female broilers has been discussed (Rawles et al., 1995). In the present study it is thought that the higher resistance of female animals might have been caused by a considerably lower diameter of the female legs. Although the feet and shackle were wetted before stunning, male broilers with thicker legs might have a tighter contact, thus improving conductivity. However, the variation is a cause for concern, as under practical conditions numerous broilers of both genders are stunned together. Multi-bird waterbath stunners might therefore cause even higher differences in current per bird, with possible effects on stunning efficiency and meat quality. Constant current stunners should therefore be considered to overcome the physical variation between broilers. The effect of different amounts of current on stunning efficiency should now be discussed.

Stunning efficiency of different waveforms

Acceptable stunning setups should induce unconsciousness in at least 90% of the animals. The occurrence of a profoundly suppressed iso-electric EEG with less than 10% of the base-line EEG has been used to determine unconsciousness and insensibility in broilers (Raj and

O'Callaghan 2004a,b; Raj et al. 2006a). In addition a sound reduction of positive responses to the corneal reflex test and spontaneous eye blinking have been identified as a tool to assess stunning efficiency (Prinz et al., 2009b,c unpublished). Figure 2 shows the effect of the different electrical waveforms on these parameters. Sine wave AC achieved the best stunning results, while application of a rectangular AC was less effective. Pulsed DC treatments showed the lowest stunning efficiency. Assessment of the development of brain waves and reflexes over time following different waveform treatments will reveal a better understanding of their effect on the induction of unconsciousness.

Sine wave alternating current

In the groups stunned with sine wave AC a low voltage of 60 V only achieved an acceptable stunning result in male broilers. Although the percentage of birds in this group with considerable brain activity in the 2-30 Hz band is slightly elevated in P1, it rapidly decreases in P2 and P3. This trend has been observed before (Prinz et al., 2009b,c unpublished) and has been interpreted with the occurrence of epileptiform activity, followed by a profound EEG reduction (Raj and O'Callaghan, 2004a,b). The results of the 13-30 Hz band however show a profound EEG suppression in 90% of the broilers in P1, which can be interpreted with insensibility to pain (Raj et al. 2006a,b). The behavioural reflexes support this conclusion, as corneal reflex and occurrence of spontaneous eye blinking are reduced to less than 30% of the animals. Female birds treated with the same constant voltage did not show a comparable reduction of brain activity and physical reflexes (Figure 2). Their stunning result was inadequate with less than 90% showing a profound suppression of the EEG in the 2-30 Hz band in all periods and in P1 also in the 13-30 Hz band. The difference might have been caused by the significantly lower stunning current of 52 mA obtained by the females compared to 72 mA for the males (Table 2). Increasing the voltage setting to 80 V delivered a similar current of 73 mA to female broilers (Table 2). This group resulted in adequate stunning efficiency with a profoundly suppressed EEG in the 2-30 and 13-30 Hz band in more than 90% of the animals (Figure 2). Moreover corneal reflex and spontaneous eye blinking could only be observed in very few animals (Figure 2). It can therefore be concluded that stunning current, not voltage has the main influence to render broiler chickens unconscious. In the present study sine wave AC of 70 mA proved sufficient to achieve unconsciousness in more than 90% of broilers. A minimum of 80 V must therefore be applied in commercial slaughterhouses to ensure adequate stunning results in female animals. Failure of resumption of breathing in the majority of birds treated with this voltage indicates the induction of cardiac arrest (Figure 2). This effect of low frequency sine wave AC has been observed before (Wilkins et al., 1998).

Rectangular alternating current

Broilers stunned with a rectangular AC showed an overall lower stunning efficiency compared to sine wave. A constant voltage of 60 V did not achieve a profound suppression of the EEG in male and female birds (Figure 2). While almost all birds resumed breathing following stunning, the majority of animals also expressed spontaneous eye blinking at 40 seconds post-stun (Figure 2). This is in contrast to the results of male broilers treated with sine wave AC of 60 V and might be caused by the lower current obtained by the broilers that received a rectangular AC (Table 2, Figure 2). When voltage is increased to 80 V, male broilers showed a profound suppression of the EEG in both the 2-30 Hz and 13-30 Hz band in more than 90% of the birds (Figure 2). However in both frequency bands P1 indicates considerable brain activity in 20% of broilers. As previously discussed this might be explained with epileptic activity or a generally deranged state of the brain caused by the current flow. Corneal reflexes and spontaneous eye blinking could only be observed in few birds (Figure 2), supporting the conclusion that this group was effectively stunned. Moreover, only 30% of the broilers were able to recover from the stunning process. Female broilers treated with the same setup were not adequately stunned according to the criteria set out in this study (Figure 2). A rectangular AC of 80 V is therefore only sufficient to render male broilers unconscious. This distinction might be caused by the lower stunning current of female broilers with 71 mA compared to 91 mA per male bird when treated with 80 V. Adequate stunning efficiency of female broilers could be achieved with 120 V rectangular AC, resulting in a current of 117 mA per animal (Figure 2). It can therefore be concluded that a minimum of 90 mA must be applied to achieve unconsciousness in 90% of the broilers treated with rectangular AC of 70 Hz. This value is considerably higher than the 70 mA minimum current established for sine wave AC. The waveform therefore has a major influence on stunning effectiveness. The more gradual rate of voltage change of a sine wave AC compared to the sharp rise of a rectangular AC has been discussed as a possible cause (Raj, 2006).

Pulsed direct current

Pulsed DC stunning seemed less effective than AC, with neither males nor females obtaining adequate stunning results with 60 or 80 V. An increase to 120 V resulted in a profound suppression of the EEG in more than 90% of male broilers for both frequency bands, 2-30 and 13-30 Hz (Figure 2). Although the percentage of animals with considerable brain power in P1 was slightly higher, this corresponds with the findings for male broilers stunned with sine wave AC of 60 Volts and rectangular AC of 80 Volts respectively and could be explained by the occurrence of epileptic activity or a deranged state of the brain caused by the current flow. The occurrence of spontaneous eye blinking could only be observed in very few animals for up to 40 seconds post-stun, supporting the conclusion of good stunning effectiveness (Figure 2). Moreover at 20 seconds post-stun the corneal reflex could not be elicited in more than 70% of the birds, indicating deep unconsciousness. In the later corneal reflex test at 40 seconds post-stun a rapid return of positive responses could be observed in animals that resumed breathing. Although spontaneous eye blinking was still markedly reduced, fast and efficient bleeding would be necessary for this stunning setup to prevent recovery of the animals before death from bleeding. Female broilers treated with the same constant voltage of 120 V however, did not show adequate stunning efficiency (Figure 2). They only received a stunning current of103 mA compared to 128 mA for males (Table 2). It can therefore be concluded that a minimum stunning current of 130 mA would be necessary to achieve unconsciousness in more than 90% of broilers treated with a pulsed DC current. This value is considerably higher than the necessary current established for sine wave or rectangular AC.

Influence of stunning time

The effectiveness of pulsed DC for waterbath stunning has been questioned before (Raj et al., 2006b,c). Raj (2006) explains the lower stunning efficiency of pulsed DC with the current flow in one direction only, whereas AC flows in positive and negative direction. Results of a previous study (Prinz et al., 2009b,c unpublished) on the other hand show no difference in stunning efficiency of pulsed DC and rectangular AC waterbath stunning. In their study a pulsed DC of 70 Hz rendered more than 90% of broilers unconscious with minimum currents of 80 mA. The distinction might be caused by the longer stunning time of 10 seconds (Prinz et al., 2009c unpublished). This might have caused a longer duration of insensibility in the broilers than four seconds stunning time in the present study. Raj et al. (2006b,c) used a shorter stunning time of one second. If the application time of a pulsed DC influences the duration of insensibility, birds might have recovered shortly after leaving the waterbath, thus leading to inadequate stunning results in the EEG analysis. The longer stunning time of 10 seconds on the other hand resulted in a longer period of insensibility with a profoundly suppressed EEG for up to 40 seconds post-stun. The influence of stunning time on the duration of unconsciousness after pulsed DC stunning needs further investigation. From the results of the present study, four seconds stunning time with a pulsed DC can only be recommended with more than 120 V for commercial slaughterhouses.

Application of AC does not show this effect of stunning time. The results presented here correspond with findings of Prinz et al. (2009b, unpublished) who analysed the effectiveness of frequency and amount of a rectangular AC on the EEG of broilers. A current of 100 mA per animal showed an adequate stunning effect when applied for 10 seconds, as compared to more than 90 mA in the present study. Raj et al. (2006a) also confirmed the effectiveness of 100 mA with up to 200 Hz sine wave AC after one second stunning time. The results presented here show an even higher effectiveness of sine wave AC of 50 Hz, where currents between 70 and 80 mA per bird achieved a profound suppression of the EEG in more than 90% of the broilers.

Epileptiform activity and clonic-tonic convulsions

The occurrence of epilepsy before the profound suppression of the EEG has been interpreted to indicate good stunning efficiency in broiler chickens (Schütt-Abraham et al., 1983). Raj et al. (2006a,b) found a high percentage of epileptiform activity in broilers stunned with AC, whereas pulsed DC resulted in fewer birds with an epileptic EEG. Prinz et al. (2009b,c unpublished) only detected epileptic activity in less than 10% of the birds with both, rectangular AC or pulsed DC. They concluded that the lower rate of epilepsy could be explained with difference in stunning time (Prinz et al., 2009b,c unpublished). While Raj et al. (2006a,b) stunned for one second, the current was applied for 10 seconds in the studies of Prinz et al. (2009b,c unpublished). Duration of epileptic activity has been reported from 9 to 17 seconds after the onset of current flow (Gregory and Wotton, 1987; Raj et al., 2006a) With 10 seconds stunning time, a considerable amount of epileptic activity might be terminated in the waterbath or during transfer of the birds to the CHEC before EEG recording (Prinz et al., 2009c, unpublished). This can be supported with findings of the present study, where epileptic activity could be detected in a considerably higher number of birds for both AC and DC stunning as compared to the previous studies (Prinz et al., 2009b,c unpublished) (Figure 4). With a shorter stunning time of four seconds, epileptic activity might have lasted for sufficient time to be recorded. Moreover a high percentage (81 to 100%) of birds showed clonic-tonic convulsions when leaving the waterbath in the present study. This has been associated with the occurrence of epilepsy in the brain (Schütt-Abraham et al.,1983). The level of epileptic activity during or shortly after waterbath stunning might therefore be considerably higher than the percentage identified on the EEG recordings.

Influence of waveforms on cardiac function

Stun to kill methods have welfare advantages as recovery of birds during bleeding is prevented (Von Wenzlawowicz and Von Holleben, 2001). Cardiac fibrillation might on the other hand cause meat quality defects (Gregory, 1989). Induction of cardiac arrest in conscious birds has been questioned due to possible welfare concerns (Raj and Tserveni-Gousi, 2000). Resumption of breathing does not necessarily coincide with sensibility, but indicates the ability of the bird to recover (von Wenzlawowicz and von Holleben, 2001). In the present study, failure of resumption of breathing following stunning was assessed as an indicator of cardiac arrest caused by the waterbath. From Figure 3 it is clear that pulsed DC showed a considerably lower effect on cardiac function than either sine wave or rectangular AC. This confirms findings of Kuenzel and Ingling (1977). However, insensibility could be achieved in broilers stunned with a pulsed DC without inducing cardiac arrest (Figure 3). This corresponds with findings of Prinz et al. (2009c, unpublished), who observed a profound suppression of the EEG following pulsed DC stunning with 70 Hz in more than 90% of the birds, although most animals resumed breathing. With increasing current levels however, birds encountered cardiac arrest. In another study on the effect of a rectangular AC, most broilers encountered cardiac arrest when stunned with 70 Hz and a minimum of 100 mA. Lower currents resulted in resumption of breathing without a profound suppression of the EEG (Prinz et al., 2009b unpublished). The present study confirms these findings, as application of a low frequency alternating current was either too low to cause a profound suppression of the EEG in 90% of the broilers, or caused cardiac arrest in the majority of birds (Figure 2).

Influence of waveforms on wing flapping

The occurrence of wing flapping in the first 40 seconds post-stun was assessed as an indicator for convulsions following cardiac fibrillation with possible effects on meat quality (Figure 4). Sine wave AC stunning caused a very high proportion of wing flapping for all three voltage settings, followed by rectangular AC (Figure 3). Pulsed DC on the other hand only resulted in a maximum of 40% wing flapping for the highest stunning voltage of 120 V (Figure 3). This level is slightly lower compared to a previous study on pulsed DC stunning of 10 seconds (Prinz et al., 2009c unpublished). It can moreover be observed that the level of wing flapping was higher in females in the present study (Figure 3). On the other hand fewer females encountered cardiac arrest, probably due to the lower stunning current. The effect of high stunning currents alone on the occurrence of wing flapping must therefore be questioned. Further studies should investigate the effect of different stunning currents and waveforms on meat quality.

CONCLUSION

The same constant voltage delivers a significantly different amount of current per broiler, depending on sex and current waveform. Female birds have a higher resistance in the electrical waterbath, independent of the life weight. This raises welfare concerns regarding multi-bird waterbaths operated at constant voltage and variable current. As stunning effectiveness is determined by stunning current, constant current stunners should be considered instead of constant voltage stunners.

The necessary stunning current to achieve unconsciousness varies significantly between waveforms. While sine wave AC showed good stunning results with 70 to 80 mA, 90 to 100 mA per bird were necessary for rectangular AC. A pulsed DC of 130 mA achieved the same effect. For pulsed DC, stunning time seems to influence the duration of unconsciousness, while this seems less important for AC. A stunning time longer than four seconds is highly recommended for commercial slaughterhouses using pulsed DC.

Low frequency alternating currents cause cardiac arrest in most birds that show adequate stunning efficiency. These setups can therefore only be recommended for stun to kill methods. Moreover the occurrence of wing flapping as an indicator for convulsions and impaired meat quality is significantly higher for AC. Pulsed DC stunning can reduce the occurrence of cardiac arrest and wing flapping. It must however be assured that birds are adequately stunned for a sufficient period to prevent recovery during bleeding. However, meat quality effects of the different stunning setups must be analysed separately.

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Chapter 7

Stunning effectiveness of broiler chickens using a two-phase stunner: pulsed direct current followed by sine wave alternating current

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ABSTRACT

Stunning efficiency of male and female broiler chickens was analysed in response to the two-phase Simmons step-up stunner. In Phase I, a pulsed DC of 550 Hz is applied in a shallow waterbath. This is immediately followed by Phase II, consisting of a metal plate with sine wave AC of 50 Hz. 120 male and female broiler chickens were randomly allocated to six stunning groups with 10 males and 10 females per group. In Phase I a voltage of 12 and 15V was applied followed by 40, 50 or 60V in Phase II. Stunning time was 10 and 5 seconds in Phase I and II respectively. The current per bird was recorded. To assess stunning efficiency the electroencephalogram (EEG) was recorded for 120 seconds post-stun. Simultaneously the occurrence of spontaneous eye blinking, breathing and wing flapping was assessed. The corneal reflex was tested every 20 seconds. The reduction of brain power in two frequency bands (2-30 Hz and 13-30 Hz) to less than 10% of the pre-stun level was analysed as indicator for adequate stunning. Female broilers showed a significantly higher electrical resistance, resulting in a lower average stunning current. Phase II showed the biggest impact on stunning efficiency. Increasing voltage improved the stunning effect, but none of the analysed treatments induced unconsciousness in at least 90% of the animals. Voltage settings of more than 60V AC in Phase II must therefore be applied. The majority of animals recovered from stunning in all groups. The occurrence of physical reflexes was suppressed in animals that were considered sensitive in the EEG analysis. Assessment of eye reflexes for the evaluation of stunning efficiency can therefore not be recommended for this stunning method. No animal showed tonic-clonic convulsions following stunning and the level of severe wing flapping was very low in all groups. Meat quality advantages of this stunning method can therefore be expected, but this must be assessed in a separate study. It must be investigated if this effect can be maintained with higher voltage settings to ensure adequate stunning efficiency.

Keywords

broiler, electroencephalogram, Simmons two-phase stunner, high frequency DC, alternating current AC

INTRODUCTION

Stunning of chickens before slaughter is mandatory in the European Union (Council Directive 93/119[2]). The most common stunning method in commercial chicken plants is an electrified waterbath. In the European Union high voltages of at least 100 V per bird are usually applied, often inducing cardiac fibrillation in the waterbath (Bilgili, 1992). Due to convulsions, this has been associated with meat quality defects such as breast muscle haemorrhaging and broken bones (Gregory and Wilkins, 1989; 1990). In the US low voltage stunning with 30 to 40 V per bird is a common method (Bilgili, 1999). A step-up stunner (Simmons Engineering Company, Dallas, GA, USA) consists of two stunning phases: In Phase I a high frequency pulsed DC with a very low voltage of 10-15 V is applied, immediately followed by Phase II with a sine wave AC of 50 Hz. The first phase renders the birds unconscious, thus avoiding muscular contractions and possible meat quality defects. In the second phase a deeper stun is induced (Simmons Engineering Company, Dallas, GA, USA). With very low currents used in both phases, broilers are able to recover from stunning. For welfare reasons it must be assured that the state of unconsciousness persists until death from bleeding supervenes (Raj, 2006).

The most objective method to assess the state of (un)consciousness is EEG analysis. The occurrence of an iso-electric, flat EEG with a profound reduction to less than 10% of the pre-stun brain power has been used to indicate unconsciousness (Raj et al., 2006). Two brain frequency bands have been considered: iso-electricity in the broader band of 2-30 Hz is indicative for overall loss of brain function, whereas the same reduction in the 13-30 Hz band has been interpreted with loss of sensibility (Raj and O'Callaghan, 2004a,b). The 'chicken EEG clamp (CHEC)' has been developed as a non-invasive method to EEGs of broiler chickens (Coenen et al., 2007). For quantitative comparison of brain power before and after stunning Prinz et al. (2009a) have analysed the typical base-line brain power of broiler chickens using the CHEC. This can now be used to calculate the relative reduction of brain power following different stunning setups. Epileptic activity with characteristic spike and wave discharges in the EEG prior to the iso-electric state has also been associated with the induction of unconsciousness in the waterbath (Schütt-Abraham et al., 1983).

[2]http://eur-lex.europa.eu

Prinz et al. (2009b,c unpublished) have analysed several physical parameters for the assessment of unconsciousness following high voltage waterbath stunning of broiler chickens. An increase of corneal reflexes together with the occurrence of spontaneous eye blinking has been identified to indicate returning consciousness. Resumption of breathing will occur in birds that did not encounter cardiac arrest during stunning but is no direct indicator for consciousness and sensibility (von Wenzlawowicz and von Holleben, 2001).Severe wing flapping seems associated with convulsions rather than returning consciousness and might therefore be an indicator for meat quality problems (Prinz et al., 2009b,c unpublished).

The aim of the present study is the assessment of the state of (un)consciousness of broiler chickens following stunning in a Simmons step-up stunner for different voltage settings in both stunning phases. Therefore the effect of the two stunning phases on the EEG and on the occurrence of physical reflexes is analysed.

MATERIAL AND METHODS

A total of 120 Ross broiler chickens, 60 males and 60 females were raised in one flock for 7 weeks. The average weight was 2.79 ± 0.21 and 2.41 ± 0.17 for males and females respectively. For stunning a Simmons step-up stunner (SF-7001 Pre-Stunner, Simmons Engineering Company, Dallas, GA, USA) consisting of two stunning phases was used. In Phase I, birds receive a pulsed DC current of 550 Hz in a shallow waterbath of about 1 cm height. The head of the chicken rests on a metal grid in the water. Water conductivity is kept constant at 40 Millisiemens/cm by the “Salt Injector Assembly” (Simmons Engineering Company). Phase II is half as long as the first phase and consists of a metal plate immediately following the waterbath. A sine wave AC of 50 Hz is applied to the chicken’s head that rests on the metal plate.

To facilitate subsequent EEG assessment, a rotating stand fixed with a grounded metal shackle was used to immerse single broilers into the stunning cabinet and to move the broilers from Phase I to Phase II. The feet of the birds were wetted before stunning to facilitate resistance break down between the legs of the animals and the shackle. Broilers were allocated to six stunning groups, each consisting of 10 male and 10 female chickens. The first three groups received a pulsed DC of 12 Volts in Phase I, with 40, 50 or 60 Volts sine wave AC in Phase II. Phase I was then increased to 15 Volts pulsed DC for the following three groups, again followed by 40, 50 or 60 Volts sine wave AC. Stunning time was 9.8 ± 0.9 seconds in Phase I and 4.9 ± 0.5 seconds in Phase II for all groups. The r.m.s current (root mean square) of every broiler was measured using a multimeter (Multimeter 189, Fluke Corp., Everett, USA) for Phase I and a scope and current probe (123 Industrial Scope Meter {20 MHz} and 80i-110s AC/DC current probe, Fluke Corp.) for Phase II. All data was recorded on to a data acquisition programme (View Forms for Phase I and View SW90W for Phase II, Fluke Corp.).

Following stunning the rotating stand swung the birds towards the CHEC. Broilers were immediately fixed into the EEG device with their feet still hanging in the shackle. The EEG recording started within 10 ± 4 seconds post-stun and lasted for 120 seconds. The EEG equipment and recording settings used in the present experiment have been described by Prinz et al. (2009b, unpublished). When exiting the stunning bath the occurrence of clonic-tonic convulsions was assessed as described by Prinz et al. (2009b,c unpublished). During EEG recording signs of breathing, spontaneous eye blinking and wing flapping were assessed and marked on observation channels on the EEG. This allows direct comparison of brain patterns with behavioural parameters. Failure of resumption of breathing was considered as a sign of cardiac arrest caused by the stunning process. The occurrence of wing flapping was regarded as an indicator for severe muscle convulsions. The corneal reflex was tested every 20 seconds post-stun and the results were recorded. Neck tension was also assessed, but due to the fixation of the chickens' head in the clamp, evaluation was difficult and this parameter has not been included in the further analysis. At the end of the recording period all birds were offered in a box filled with carbon dioxide.

For analysis, all EEG records were transferred to an analyzer (Brainvision Analyzer, Brain Products, 82205 Gilching, Germany www.brainproducts.com) using a Software-aid to convert Windaq-data (Dataq Instruments, Inc., Akron, OH. USA. www.dataq.com). The traces were filtered for the broader brain frequency band of 2-30 Hz and the smaller frequency band of 13-30 Hz and subdivided into three post-stun periods: P1 0-20 seconds, P2 20-30 seconds and P3 30-40 seconds. In each period, five segments of one second were marked and a Fast Fourier Transformation (FFT) calculated. From the Grand Average of the five segments, the total brainpower for each of the three post-stun periods was determined. This was expressed as a percentage of the base-line brainpower of a wake broiler chicken (Prinz et al., 2009a). The relative brain power following the different stunning treatments was used to evaluate the level of (un)consciousness. A profound reduction of total brainpower to less than 10% of the base-line EEG power was interpreted to be equivalent to an iso-electric flat EEG. A profoundly suppressed, iso-electric EEG in the 2-30 Hz band indicates failure of overall brain function, whereas the same reduction in the 13-30 Hz band was considered to be indicative for loss of sensibility. If birds showed a relative brainpower of more than 10% of the base-line EEG they were considered to be inadequately stunned. In a visual assessment of the EEG recordings the occurrence of epiletiform activity was marked where the traces showed typical low frequency (2-6 Hz) spike and wave discharges (Figure 1). A characteristic chaotic EEG pattern with high amplitude and low frequency directly after stunning, followed by an iso-electric EEG could be observed in many birds. This was also regarded as an indicator for a form of unconsciousness.

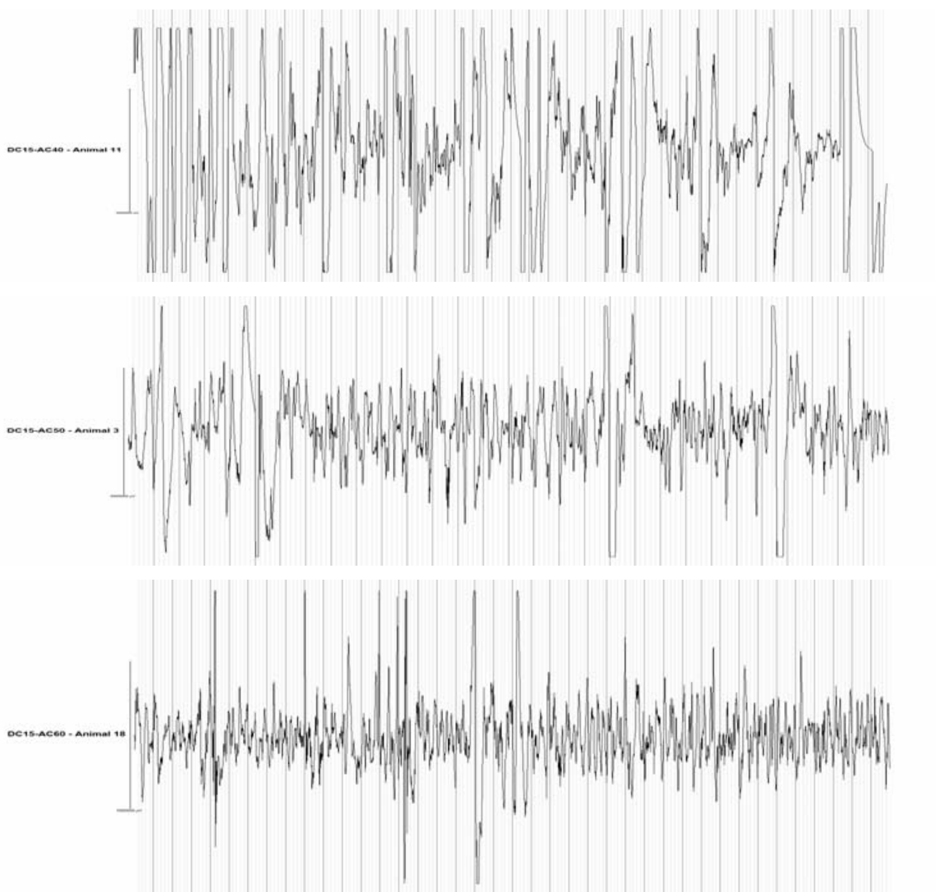


Figure 1. Examples of EEG traces of broiler chickens following stunning with the Simmons step-up stunner with three different voltage setting in Phase II sine wave AC: 40 V in the upper panel, 50 V in the middle panel and 60 V in the lower panel. The amplitude of the Y-axis is 80 µV and the area between the bold vertical lines represent 1 second.

For statistical analysis a Nominal Logistic Regression was conducted using JMP 7.1 (2007). All factor effects were calculated with the Chi-squared [χ^2] Likelihood Ratio Test with Phase I Voltage [V1], Phase II Voltage [V2], sex and the interaction of V1 x V2, V1 x sex, V2 x sex and V1 x V2 x sex as fixed factors (Table 1). From the predicted values the likely percentage of birds not showing an iso-electric EEG or expressing positive behavioural reflexes was obtained and plotted.

RESULTS

In both stunning phases, voltage and sex had a significant influence on the current per bird (Table 1). Male broilers obtained a significantly higher stunning current when stunned with the same voltage as compared to female broilers (Figure 2). This was not caused by deviation in life weight, as the statistical analysis was corrected for this factor. With increasing voltage the effective stunning current increased in both stunning phases.

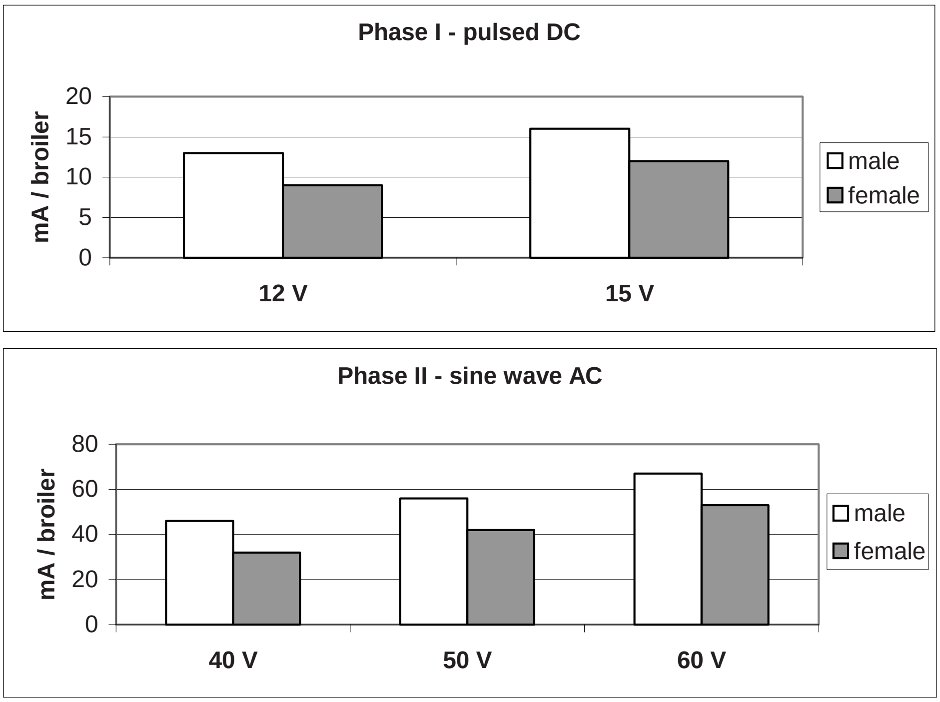


Figure 2: Average current obtained by male and female birds in both stunning phases. Phase 1: pulsed DC of 550 Hz at constant voltage of 12V or 15V. Phase 2: sine wave AC of 50 Hz at constant voltage of 40, 50 or 60V.

A total of 100 EEG traces were submitted to Fast Fourier Transformation (44 for males and 56 for females). 20 records could not be included due to movement artefacts and disturbances. The EEG analysis showed a significant effect of stunning voltage in Phase II (V2 AC) for the occurrence of an iso-electric EEG in the first 40 seconds post-stun in both brain frequency bands, the broader band of 2-30 Hz and the more limited band of 13-30 Hz (Table 1). With increasing V2 AC voltage, significantly more birds obtained an iso-electric EEG (Figure 3).

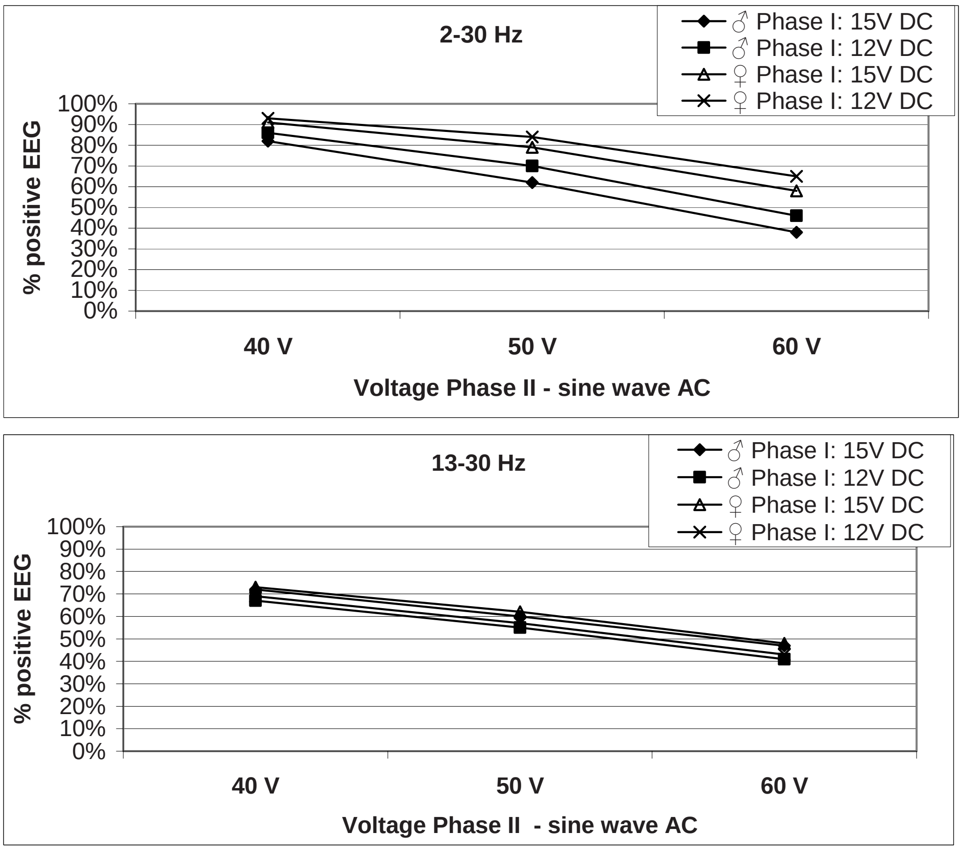


Figure 3. Percentage of male and female birds not showing an iso-electric EEG (less than 10% of the base-line EEG) in the first 40 seconds post-stun, in response to the voltage obtained in stunning Phase I and stunning Phase II. The upper panel shows the results of the 2-30 Hz band, the lower panel contains the outcome of the 13-30 Hz band.

For the 2-30 Hz band representing overall brain function, the interaction of voltage in Phase I (V1 DC) x sex showed a significant influence, whereas this was not significant for the 13-30 Hz band. When stunned with the same DC voltage in Phase I, significantly more male broilers showed an iso-electric EEG in the 2-30 Hz band as compared to females (Figure 3).

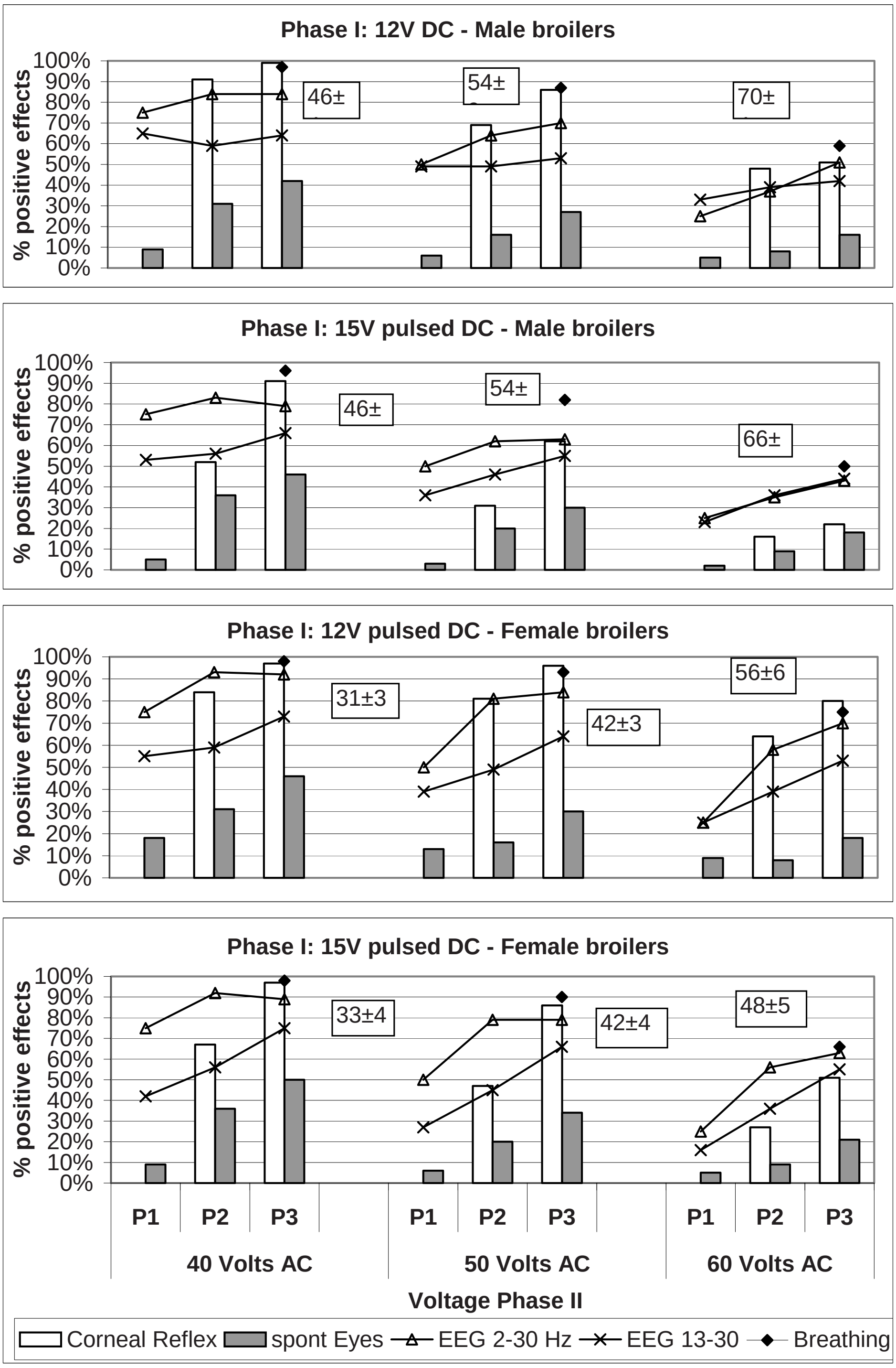
Table 1. Results of the statistical analysis: Nominal Logistic Regression

Factor Effects	**Voltage Phase I [V1 DC]**	**Voltage Phase II [V2 AC]**	**Sex**	**V1 DC x Sex**	**V2 AC x Sex**	**V1 DC x V2 AC**	**V1 DC x V2 AC x Sex**
Effective current Phase 1	p<0.0001		p<0.0001	n.s.			
Effective current Phase 2		p<0.0001	p<0.0001		n.s.		
EEG 2-30Hz (P1-P3)	n.s.	p=0.0003	n.s.	p=0.0095	n.s.	n.s.	n.s.
EEG 13-30 Hz (P1-P3)	n.s.	p=0.0304	n.s.	n.s.	n.s.	n.s.	n.s.
EEG P1 2-30 Hz	n.s.	p=0.0007		p=0.0065	n.s.	n.s.	n.s.
EEG P1 13-30 Hz	n.s.	p=0.025	n.s.	n.s.	n.s.	n.s.	n.s.
EEG P2 2-30 Hz	n.s.	p=0.0006	n.s.	n.s.	n.s.	n.s.	n.s.
EEG P2 13-30 Hz	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EEG P3 2-30 Hz	n.s.	p=0.0057	n.s.	p=0.0192	n.s.	n.s.	n.s.
EEG P3 13-30 Hz	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Corneal Refl. 20 sec	p<0.0001	p=0.0004	n.s.	n.s.	n.s.	n.s.	n.s.
Corneal Refl. 40 sec	p=0.002	p<0.0001	n.s.	n.s.	n.s.	p=0.0207	n.s.
Spont. Eyes P1	n.s.	n.s.	n.s.	n.s.	p=0.0031	n.s.	n.s.
Spont. Eyes P2	n.s.	p=0.0004	n.s.	p=0.0135	p=0.012	n.s.	p=0.0498
Spont, Eyes P3	n.s.	p=0.0027	n.s.	p=0.0435	p=0.0036	p=0.0012	n.s.
Breathing	n.s.	p<0.0001	n.s.	p=0.0298	n.s.	n.s.	n.s.
Wing flapping	n.s.	n.s.	n.s.	n.s.	n.s.		
Epil. activity	p=0.0385	n.s.	p=0.0122	p=0.0292	n.s.	n.s.	n.s.

In the three post-stun periods P1, P2 and P3, AC voltage in Phase II showed a significant effect on the occurrence of an iso-electric EEG in the 2-30 Hz band. With increasing AC voltage significantly more birds obtained a flat EEG (Figure 4). In the 13-30 Hz band this effect could only be confirmed in P1 (Table 1). In addition the interaction of V1 DC x sex showed a significant effect for the 2-30 Hz band in P1 and P3. When stunned with the same DC voltage in Phase I, significantly more male broilers obtained an iso-electric EEG compared to female birds (Figure 4).

The corneal reflex test at 20 and 40 seconds post-stun was significantly influenced by V1 DC and V2 AC (Table 1). Both, male and female broilers showed significantly less corneal reflexes with increasing voltage. For the later test at 40 seconds post-stun the interaction of V1 DC x V2 AC showed a significant effect (Table 1). The occurrence of spontaneous eye blinking was significantly influenced by several factors. In all three post-stun periods a significant effect of the interaction of V2 AC x sex could be detected (Table 1). The interaction of V1 DC x sex was significant in P2 and P3 (Table 1). With the same stunning voltage male broilers showed less spontaneous eye blinking than females (Figure 4). Increasing V2 AC significantly suppressed the occurrence of spontaneous eye blinking in P2 and P3 for all animals (Figure 4). In P2 the interaction of V1 DC x V2 AC was significant, and in P3 the interaction of all three factors V1 DC xV2 AC x sex proved significant (Table 1). Resumption of breathing was significantly influenced by V2 AC, fewer birds showed signs of breathing with increasing voltage (Table 1 and Figure 4). The interaction of V1 DC x sex also showed a significant effect (Table 1). When stunned with the same voltage in Phase I, more female broilers resumed breathing following stunning compared to males (Figure 4).

Right Page: Figure 4. Percentage of birds not showing an iso-electric EEG (<10% pre-stun power) and percentage of birds with positive reflexes: corneal reflex, breathing and spontaneous eye blinking in different periods post-stun: P1 0-20 seconds, P2 20-30 seconds, P3 30-40 seconds. Numbers in the boxes show average effective current in the groups and standard deviation.


Phase I: 12V DC - Male broilers
46±
54±
70±
Phase I: 15V pulsed DC - Male broilers
46±
54±
66±
Phase I: 12V pulsed DC - Female broilers
31±3
42±3
56±6
Phase I: 15V pulsed DC - Female broilers
33±4
42±4
48±5
% positive effects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
P1 P2 P3 P1 P2 P3 P1 P2 P3
40 Volts AC
50 Volts AC
60 Volts AC
Voltage Phase II
Corneal Reflex
spont Eyes
EEG 2-30 Hz
EEG 13-30
Breathing

The level of wing flapping was very low in all groups and no significant difference could be detected (Table 1 and Figure 5). No bird showed tonic-clonic convulsions when leaving the waterbath, all animals had a relaxed body.

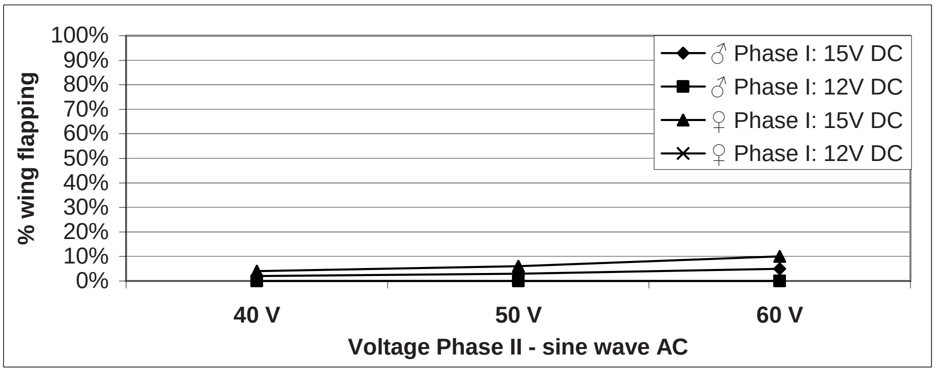


Figure 5. Percentage of wing flapping in the different stunning setups.

A significant effect of V1 DC, sex and the interaction of V1 DC x sex could be detected for the occurrence of epileptic activity (Table 1). More female broilers expressed epileptiform activity in the EEG compared to males. Higher voltage in Phase I suppressed epileptic activity (Figure 6).

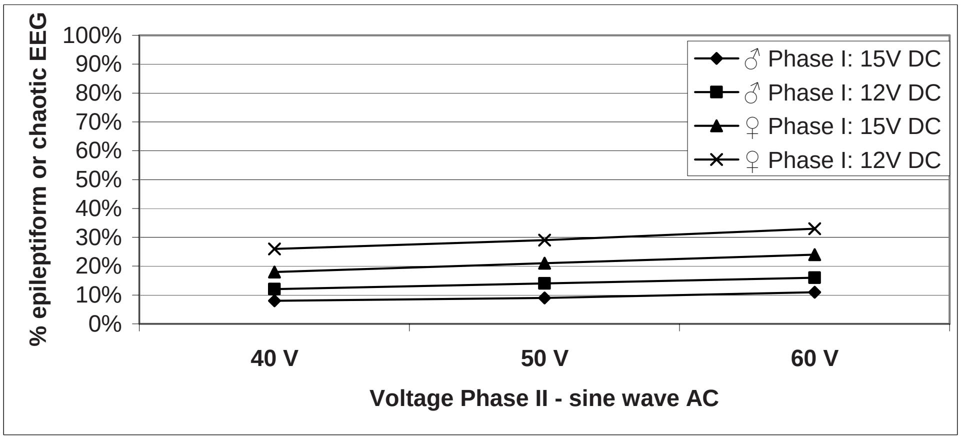


Figure 6. Occurrence of an epileptiform or chaotic EEG (low frequency epilepsy and slow wave disturbance) in the different stunning groups.

DISCUSSION

The aim of the study was the assessment of the state of (un)consciousness of broiler chickens following stunning in a Simmons step-up stunner with different voltage settings in both phases.

Electrical resistance

While the voltage was kept constant within the stunning groups, female broilers obtained a significantly lower stunning current in both stunning phases indicating a higher electrical resistance (Table 1 and Figure 2). As data was corrected for life weight in the statistical analysis, this effect was not caused by the deviation in life weight. The high conductivity in the waterbath in Phase I, controlled by the Salt Injector Assembly (Simmons Engineering Company, Dallas, GA, USA), could not eliminate this effect. Moreover the higher resistance could also be observed in Phase II, consisting of a metal plate with direct contact to the chicken’s head. This leads to the conclusion that the transition resistance between head and life electrode did not cause the significant difference. Prinz et al. (2009d, unpublished) observed a similar effect in a study on waterbath stunning with different electrical waveforms. They suggested the transition resistance between broiler feet and shackle to be responsible for the marked distinction. This might be caused by the smaller leg diameter of females, resulting in a weaker contact to the shackle (Prinz et al., 2009d unpublished). Application of water spray to wet feet and shackle before stunning could not eliminate this effect. Alternatively, the higher content of abdominal fat with very low conductivity in female broilers has been discussed as a reason for the higher resistance (Rawles et al., 1995). The variation causes welfare concerns, as under commercial conditions male and female birds are stunned together in a multi-bird stunner. This may cause even greater deviations of current obtained by single animals. The influence on stunning efficiency will now be discussed.

Assessment of stunning efficiency using EEG analysis

The analysis of stunning effectiveness was limited to the first 40 seconds post-stun, as the stunning process must ensure complete unconsciousness until death from bleeding supervenes. According to the instructions manual of the Simmons Pre-Stunner the sine wave AC in Phase II is responsible for a thorough stun, preventing movement of the birds during bleeding (Simmons Engineering Company, Dallas, GA, USA). This could be confirmed in the present study. Increasing voltage in Phase II resulted in significantly more birds obtaining a profoundly suppressed, iso-electric EEG with less than 10% of the base-line brainpower in both brain frequency bands, 2-30 Hz and 13-30 Hz (Figure 3). Voltage in Phase II therefore proved mainly responsible for the induction of unconsciousness. However, with the highest voltage setting of 60 V in the present study, more than 40% of the broilers showed inadequate stunning results in both frequency bands. This stunning setup can therefore not be recommended and further assessment of higher voltage settings is necessary to establish a minimum stunning current that ensures a stunning efficiency of 90% of the animals.

The voltage setting in Phase I did not show a significant influence on the induction of unconsciousness in the EEG (Table 1). However, in the 2-30 Hz band both, male and female broilers, showed slightly more iso-electric EEGs with the higher voltage setting of 15 V pulsed DC in Phase I, compared to the lower setting of 12 V pulsed DC (Figure 3). A further increase of voltage in Phase I might therefore improve stunning efficiency. It should moreover be considered that the effect of Phase I might be more pronounced with a greater difference of voltage between treatments. In the present study the instructions of the stunner manual (Simmons Engineering Company, Dallas, GA, USA) were applied. However the effect of a higher voltage in Phase I on the induction of unconsciousness should be investigated.

Two stunning phases

The Simmons step-up stunner is usually used in combination with very quick and efficient bleeding (Simmons Engineering Company, Dallas, GA, USA). From the results of the present study it can however be observed that stunning efficiency is not sufficient even in the first 20 seconds post-stun (Figure 4). Boilers stunned with 60V sine wave AC in Phase II show the best results with 75% of the animals obtaining iso-electricity in the EEG in P1. However, the birds show a quick recovery in the EEG. This is in contrast to findings of Prinz et al. (2009d, unpublished) on broilers stunned with 60V sine wave AC of 50 Hz in a single stunning phase for four seconds. In their study more than 90% of the male birds and 89% of the female broilers maintained a profoundly suppressed iso-electric EEG for up to 40 seconds post-stun. The average stunning current was 72 mA and 52 mA for male and female animals respectively (Prinz et al., 2009d unpublished), thus similar to the average current in the present study (Figure 4). Moreover only 20% of the male broilers in their study recovered from stunning, whereas in the present experiment 50-60% of the males resumed breathing (Figure 4). It can be assumed that this is caused by the application of the high frequent DC in Phase I, but the physiological reason remains unclear.

Evaluation of physical reflexes

Positive responses to the corneal reflex test were markedly decreased with a higher stunning voltage in Phase I (Figure 4). The occurrence of spontaneous eye blinking was very low in all groups with considerably less than 10% in P1 (Figure 4). Prinz et al. (2009d, unpublished) found 14% of spontaneous eye blinking in P1 in well stunned broilers after application of 60V sine wave AC of 50 Hz in a single phase. It can therefore be assumed that application of a high frequency pulsed DC in Phase I suppresses physical reflexes. In similar studies on single phase, high voltage stunning using either AC or DC currents, corneal reflexes and spontaneous eye blinking returned sooner than brain activity in recovering birds (Prinz et al., 2009b,c,d unpublished). It was therefore concluded that the occurrence of eye blinking indicates beginning recovery after stunning. In the present study however, the percentage of birds without iso-electric EEG is considerably higher than the number of animals expressing spontaneous eye blinking in all groups (Figure 4). This is obvious in the group of male broilers stunned with 15V pulsed DC followed by 60V AC. Although both EEG frequency bands indicate brain activity and sensibility in 45% of the animals, only 22% and 18% of the birds show a positive corneal reflex and spontaneous eye blinking respectively (Figure 4). It must be concluded that potentially conscious birds do not express physical reflexes and their assessment can therefore not be recommended to evaluate stunning effectiveness of the Simmons stunner. It can be assumed that the suppression of physical reflexes is influenced by the low voltage pulsed DC in Phase I. However the effect of the two stunning phases on the induction of unconsciousness and the physical appearance of the animals should be further investigated.

Epileptiform activity

An epileptic EEG following waterbath stunning has been used as an indicator for unconsciousness (Schütt-Abraham et al., 1983). Raj et al. (2006) found epilepsy in 90% of broilers stunned with a high voltage AC for one second. Prinz et al., (2009d unpublished) reported a lower prevalence of epileptic activity (maximum 40%) following four seconds stunning time with sine wave AC of 50 Hz. Their findings correspond with the results of the present study, where a maximum of 30% of the birds showed epileptic activity (Figure 6) with a lower percentage in male broilers compared to females (Prinz et al., 2009d unpublished). The lower occurrence of epileptic activity of the present study compared to the findings of Raj et al. (2006) might be caused by the longer stunning time. Some epileptiform activity might have occurred already in the waterbath or during transfer of the birds to the CHEC (Prinz et al., 2009b,c,d unpublished). Considering that the level of epileptic activity in the present study is similar to the results of single phase AC or DC stunning, it can be concluded that both stunning systems have a similar effect on the occurrence of epilepsy.

Absence of wing flapping and convulsions

The Simmons step-up stunner aims to prevent muscle convulsions, which could lead to impaired meat quality (Simmons Engineering Company, Dallas, GA, USA). Indeed in the present study, the bodies of the birds were relaxed with no bird showing clonic-tonic convulsions when leaving the waterbath. This is in contrast to single phase stunning with sine wave AC of 60 V, where 75% of the birds showed tonic convulsions (Prinz et al.2009d unpublished). Severe wing flapping has been interpreted to indicate convulsions (Prinz et al., 2009b,c unpublished). After single-phase sine wave AC stunning of 60V, 40 to 60% of the birds expressed severe wing flapping within 40 seconds post-stun (Prinz et al., 2009d unpublished), compared to less than 10% in the experiment presented here (Figure 5). It can be assumed that application of a low voltage pulsed DC in Phase I reduces the occurrence of convulsions. This might have a positive effect on meat quality and should be investigated in a separate study. It must however be taken into consideration that the suppression of the undesired muscular reflexes occurs already in response to electrical setups, which do not produce unconsciousness. Further studies are required to investigate whether adequate stunning can be combined with low levels of muscular reflexes.

CONCLUSION

The voltage settings tested in the present study did not achieve unconsciousness in a sufficient number of birds. Voltages higher than 60V AC in Phase II must therefore be applied. Application of a low voltage DC in Phase I showed a suppressing effect on all physical reflexes. A number of birds with considerable brain activity in the EEG analysis did not show a corneal reflex or spontaneous eye blinking. Assessment of physical reflexes can therefore be misleading for the evaluation of stunning effectiveness using the Simmons step-up stunner. Moreover the state of (un)consciousness directly following the low voltage pulsed DC in Phase I should be investigated, to ensure complete insensibility.

The two-stage Simmons stunner considerably reduces convulsions and cardiac fibrillation induced by the waterbath, which are often seen for single phase stunning using sine wave AC. The low percentage of wing flapping following stunning indicates the absence of convulsions.

This might have a positive effect on meat quality. It should be investigated if this positive effect can be maintained with higher voltages to ensure adequate stunning.

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Chapter 8

General Discussion

The aim of the study was the evaluation of a range of electrical stunning setups applied in an electrified waterbath to understand the effect of current, frequency and waveform and their interrelationships on the induction of unconsciousness in male and female broiler chickens. EEG analysis as well as physical reflexes have been used to determine the state of (un)consciousness.

The development of the ‘chicken EEG clamp-CHEC’ (Coenen et al., 2007) facilitated the analysis of a great number of broilers under the same experimental conditions. The application of external EEG electrodes has been validated with broilers under three different types of anaesthesia and has proved to be successful for brain wave recording (Coenen et al., 2007). The typical brain wave pattern recorded from the anaesthetised broilers, each specific for the type of drug used, clearly proved the reliability of the CHEC for brain wave recording of broilers. This provided the opportunity to analyse EEGs following waterbath stunning with a wide range of electrical setups, without the laborious and potentially stressful implantation of EEG electrodes into the broilers’ brains.

For the evaluation of unconsciousness, the occurrence of a profoundly suppressed iso-electric EEG with less than 10% of the pre-stun brainpower has been used (Raj and O’Callaghan 2004a,b). Construction of the typical base-line EEG, derived from the EEG of 15 wake broiler chickens, facilitated a comparative assessment of all stunning groups, related to the same wake broiler EEG (Prinz et al., 2009a). Consequently, a large-scale experiment was carried out, using alternating and pulsed direct currents ranging from 60 to 150 mA and frequencies ranging from 70 to 1500 Hz. Moreover the effectiveness of sine wave AC, rectangular AC and pulsed DC has been assessed at low frequency and constant voltage settings of 60, 80 and 120 Volts. The Simmons step-up stunner has been tested with a low voltage of 12 and 15 Volts pulsed DC in Phase I followed by 40 to 60 Volts sine wave AC in Phase II.

Effectiveness of rectangular alternating and pulsed direct current

Both rectangular AC and pulsed DC stunning of broilers were successful to render broiler chickens unconscious with a profoundly suppressed EEG for at least 40 seconds post-stun (Prinz et al., 2009b,c unpublished). Stunning efficiency was mainly influenced by the electrical frequency with both waveforms. It is obvious that frequencies above 400 Hz are not effective and cannot be recommended with a maximum current of 150 mA. In a considerable percentage of broilers stunned with 800 or 1500 Hz rectangular AC or pulsed DC a profoundly suppressed EEG could not be achieved even with the highest stunning current of 150 mA (Prinz et al., 2009b,c unpublished). For the application of a rectangular AC, the amount of current showed a significant influence (Prinz et al., 2009b unpublished). Broilers stunned with current levels below 100 mA did not obtain a profoundly suppressed EEG in at least 90% of the birds and can therefore not be recommended for rectangular AC stunning. This corresponds with the results of Raj et al. (2006a). When a pulsed DC was applied however, only the interaction current x frequency showed a significant effect (Prinz et al., 2009c, unpublished). All tested stunning currents of 80, 100, 120 and 150 mA achieved a profound reduction of the EEG to less than 10% of the base-line EEG in at least 90% of the broilers when applied with a maximum frequency of 200 Hz. A stunning frequency of 400 Hz achieved an adequate stunning efficiency only with a minimum current of 150 mA. The good stunning results of pulsed DC are in contrast to findings of Raj et al. (2006b,c). In the present study the lower stunning efficiency of a pulsed DC was confirmed in a direct comparison of the three waveforms sine wave AC, rectangular AC and pulsed DC at low frequencies with a stunning time of four seconds. In this particular experiment pulsed DC did not achieve a profound EEG reduction in more than 90% of the animals even with a higher stunning current of 130 mA (Prinz et al., 2009d, unpublished). Considering the results described previously this is surprising and it can only be concluded that the effectiveness of pulsed DC stunning depends on the application time of the current. One second applied by Raj et al. (2006b) did not result in adequate stunning, even with a high current of 150 mA. With four seconds stunning time a minimum current of 130 mA was established with a frequency of 70 Hz (Prinz et al., 2009d, unpublished). A longer stunning time of 10 seconds pulsed DC however, resulted in good stunning efficiency with a minimum current of 80 mA and a maximum frequency of 200 Hz (Prinz et al., 2009c, unpublished).

The effect of the waveform on the minimum necessary stunning current for the induction of unconsciousness is apparent from the comparison of sine wave AC, rectangular AC and pulsed DC at low frequencies (Prinz et al., 2009d unpublished). For sine wave AC, a minimum current of 70 to 80 mA was established, while a rectangular waveform required slightly higher currents of 90 to 100 mA. The higher stunning efficiency of the sine wave treatments has been associated with the more gradual rate of voltage change compared to the rectangular waveform (Raj, 2006). Pulsed DC showed the lowest stunning efficiency. This has been explained with the excursion of the electrical voltage in one direction only (Raj, 2006). However, the influence of stunning time has already been discussed.

Epileptiform activity

The main argument of Raj et al. (2006b,c) for the disapproval of a pulsed DC is the fact that the broilers failed to develop epileptic activity following stunning. The occurrence of epilepsy before the profound reduction of the EEG has been used as an indicator for effective stunning (Schütt-Abraham et al., 1983). In the present study the level of epileptiform activity was considerably lower in all stunning groups compared to Raj et al. (2006a,b,c), with a maximum of 20% and 50% for AC and pulsed DC respectively (Prinz et al., 2009b,c unpublished). Raj and O'Callaghan (2004a) reported an average duration of epileptic activity of 9 to 12 seconds after a one second stunning time. In a study of Gregory and Wotton (1987) epilepsy lasted for 17 and 12 seconds after the onset of current flow. With a stunning time of 10 seconds in the present study, EEG recordings started within 18 to 20 seconds after the beginning of current flow and epileptic activity could therefore have terminated before EEG recording (Prinz et al., 2009b,c). This assumption is supported by the fact that the EEG recordings of the groups stunned for four seconds using a low frequency AC or pulsed DC showed a considerably higher occurrence of epileptiform activity when compared to the same frequency setup with a 10 seconds stunning time (Prinz et al., 2009b,c,d unpublished). Alternatively, the animals might have passed into a state of sudden neuronal death (Raj et al., 2006b) and the occurrence of epileptic activity may not necessarily be required to prove unconsciousness. The profound reduction of brainpower clearly indicates adequate stunning efficiency of both AC and DC, disregarding of a lower rate of epileptic activity.

Effectiveness of the two-phase stunner

The Simmons step-up stunner combines both waveforms in two stunning phases. A high frequency pulsed DC of 12 to 15 Volts for 10 seconds in Phase I is immediately followed by a 50 Hz sine wave AC of 40 to 60 Volts for five seconds in Phase II. EEG analysis of the groups stunned with a Simmons step-up stunner revealed considerably different results in comparison to the stunning treatments described before. The application of two stunning phases did not achieve iso-electricity in at least 90% of the birds, even with the highest voltage level of 60 V AC in Phase II (Prinz et al., 2009e unpublished). This is surprising, as a sine wave AC of 60 V achieved good stunning results in male broilers when applied in a single phase for four seconds (Prinz et al., 2009d unpublished). It must be concluded that the low voltage pulsed DC in Phase I causes this distinction, but the physiological reason remains unclear. From the results presented here it can be assumed that a higher voltage setting in Phase II would improve stunning effectiveness (Prinz et al., 2009e unpublished).

Influence of electrical waveforms on cardiac function

Stun to kill methods have been associated with welfare advantages, as recovery of the animals during bleeding is prevented (Gregory and Wotton, 1987). It is however not necessary, provided the state of unconsciousness persists until death from bleeding supervenes (von Wenzlawowicz and von Holleben, 2001). In the present study, failure to resumption of breathing following waterbath stunning was interpreted with the occurrence of cardiac arrest. In the lowest acceptable AC stunning setup of 100 mA and a maximum of 200 Hz, the majority of birds encountered cardiac arrest (Prinz et al., 2009b unpublished). DC resulted in good stunning efficiency with a lower current of 80 mA and maximum frequencies of 200 Hz, while up to 80% of the birds resumed breathing within 15 seconds post-stun (Prinz et al., 2009c unpublished). This effect of DC stunning has been observed before (Kuenzel and Ingling, 1977). From the direct comparison of the three waveforms it could be assumed that induction of unconsciousness with AC at low frequency coincides with cardiac arrest. Good stunning results could only be obtained in groups where at least 80% of the animals encountered cardiac arrest. Pulsed DC stunning on the other hand achieved a profoundly suppressed EEG in at least 90% of the broilers with a survival rate of 50%, although a higher overall current level was necessary (Prinz et al., 2009d, unpublished). It could therefore be concluded that induction of unconsciousness with AC at low frequency is usually connected to cardiac arrest stunning, whereas application of a pulsed DC can render broiler chickens unconsciousness with a smaller effect on cardiac function. This is supported by the results of the Simmons stunner (Prinz et al., 2009e unpublished): a constant voltage of 60V sine wave AC did not achieve a profound reduction of the EEG in at least 90% of male broilers when a 550Hz pulsed DC of 15 V was applied before. The majority of animals resumed breathing in this stunning treatment. The same constant voltage of 60 V AC resulted in more than 90% iso-electric EEGs in males when applied in a single phase only (Prinz et al., 2009c unpublished). 80% of the animals in this group did not recover from stunning. AC stunning with higher frequencies can reduce the effect on cardiac function and nevertheless achieve good stunning results. Application of a rectangular AC of 400 Hz with 120 or 150 mA resulted in adequate stunning effectiveness and resumption of breathing in 80 and 65% respectively (Prinz et al., 2009b unpublished). The results of both, AC and DC stunning however indicate that induction of unconsciousness in at least 90% of the animals cannot be achieved with a stunning setup that ensures a 100% survival rate (Prinz et al., 2009b,c unpublished). This causes concerns for ritual slaughter, where the animals must be alive at the time of neck cutting.

Eye reflexes as indicators for stunning efficiency

Eye reflexes proved to be most suitable as indicators for the state of unconsciousness. The corneal reflex is tested by touching the cornea with a feather, which provokes blinking of the nictating membrane (Gregory, 1989). A positive response has been associated with a beginning recovery of brain functions, not necessarily related to consciousness (von Wenzlawowicz and von Holleben, 2001). This was confirmed in the present studies. The corneal reflex returned soon in broilers that resumed breathing (Prinz et al., 2009b,c,d unpublished). However it could be elicited in a considerable number of broilers that showed an iso-electric EEG, thus unconsciousness. It is well known that the corneal reflex is a brain stem reflex, which is present under general anaesthesia. It can therefore be concluded that positive responses to the corneal reflex test do not necessarily indicate sensitivity in broilers, whereas absence of corneal reflexes clearly indicates deep unconsciousness or approaching death (Gregory, 1989). A high percentage of positive responses or a sharp increase of corneal reflexes over time indicates a rapid recovery of the animals. Comparing the EEG results with the occurrence of corneal reflexes in the present study, a maximum of 30% of corneal reflexes can be accepted, while the EEG indicates adequate unconsciousness (Prinz et al., 2009b,c,d unpublished).

Spontaneous eye blinking has been considered as an indicator that might be more related to returning consciousness. Suppression of this reflex in response to stunning is clearly shown in the present studies (Prinz et al., 2009b,c,d unpublished). However, in groups with good stunning efficiency according to the EEG analysis, up to 15% of broilers expressed spontaneous eye blinking within the first 20 seconds post-stun. Moreover some birds treated with an AC showed spontaneous eye blinking although they did not resume breathing. In these cases the reflex cannot be related to recovery, but might be caused by muscular fibrillation induced by approaching death. This assumption is supported by the fact that spontaneous eye blinking occurred at a very high frequency and terminated abruptly after a few seconds. Recovering birds on the other hand continued eye blinking at longer, regular intervals for the complete recording period. Under practical conditions a distinction between conscious blinking and blinking as a result of muscle vibrations is very difficult. It can however be concluded that the absence of spontaneous eye blinking clearly indicates deep unconsciousness, while a sharp increase is a sign for rapid recovery. Since a small percentage of spontaneous eye blinking can occur in well-stunned birds, a level of 15% and 30% at 20 and 30 seconds post-stun respectively may be acceptable.

Impact of the two-phase stunner on physical reflexes

The occurrence of eye reflexes following stunning with a Simmons step-up stunner showed different results (Prinz et al., 2009e unpublished). Very few broilers expressed spontaneous eye blinking or corneal reflexes in treatments where the EEG analysis indicated inadequate stunning. This becomes most apparent in groups stunned with a higher voltage pulsed DC in Phase I. Although 45% of the males treated with 15 V pulsed DC followed by 60V sine wave AC show a relatively high brain activity, not even half of these animals show a corneal reflex or spontaneous eye blinking. In contrast to single phase AC or DC stunning, eye reflexes are no suitable indicators to assess unconsciousness in broilers stunned with a Simmons step-up stunner. Potentially conscious and sensitive animals do not necessarily express eye reflexes with this stunning method and would therefore be judged to be well stunned. It is assumed that application of a high frequent, low voltage pulsed DC in Phase I causes the suppression of physical reflexes, but the physiological mechanisms remain unclear. Further studies would be necessary to reveal the effect of the combination of pulsed DC and sine wave AC in two consecutive phases on the chickens’ brain and body.

Wing flapping as indicator for convulsions

The occurrence of wing flapping has been assessed as an indicator for returning consciousness. The present results, however, suggest that it is more associated with convulsions due to stunning. The percentage of wing flapping is increased in those groups, where the majority of birds encountered cardiac arrest (Prinz et al., 2009b,c,d unpublished). Moreover wing flapping usually occurred within the first 40 seconds post-stun, whereas this could not be observed in a later period, when the birds had fully recovered. If vigorous wing flapping is caused by convulsions, it can have negative effects on meat quality. Application of a pulsed DC resulted in a slightly lower level of wing flapping than AC and might therefore be preferential for good meat quality (Prinz et al., 2009b,c,d unpublished). It is however surprising that more wing flapping could be observed in female broilers, although they received a lower effective current compared to males (Prinz et al., 2009d unpublished). It must therefore be questioned if the amount of current alone has an influence on the occurrence of convulsions. The effect of the different stunning setups on meat quality must certainly be assessed in a separate study.

The results of the Simmons step-up stunner show a clear reduction of muscular reflexes. Less than 10% of the birds across all stunning groups showed severe wing flapping and no animal showed tonic-clonic convulsions (Prinz et al., 2009e unpublished). This confirms the information provided by Simmons. The absence of convulsions facilitates automatic cutting and improves carcass quality (Simmons Engineering Company, Dallas, GA, USA). It must however be taken into consideration that the suppression of the undesired muscular reflexes occurs already in response to electrical setups, which do not produce unconsciousness. Further studies are required to investigate whether adequate stunning can be combined with low levels of muscular reflexes.

Different effects for male and female broilers

The second aim of the study was the assessment of differences between male and female broilers, regarding the induction of unconsciousness. Rectangular AC stunning with a constant current of several frequencies did not reveal any significant differences (Prinz et al., 2009b unpublished). Application of a pulsed DC with the same range of electrical frequencies proved to be less effective in females in the EEG analysis, while on the other hand they expressed less physical reflexes (Prinz et al., 2009c unpublished). Raj and O'Callaghan (2004 a,b) observed an increase in overall brain power in a period directly following stunning. This has been explained with the occurrence of epileptiform activity. In the present study the difference between males and females could only be observed in P1 (0-20 seconds post-stun). It could therefore be caused by a slightly higher incidence of epileptic activity in females, resulting in a higher initial level of post-stun brainpower. This would cause a better stunning efficiency and therefore explain the absence of physical reflexes. Although the physiological reason remains unclear, it must be considered that in both studies voltage was adjusted to deliver the same current to all birds in a stunning group, leading to a considerably higher voltage for females (Prinz et al., 2009b,c unpublished).

The difference in electrical resistance between males and females was shown by Prinz et al. (2009d, unpublished). To simulate commercial slaughterhouses conditions, both males and females were stunned with the same constant voltage. Females received a significantly lower effective stunning current in all groups, even when the statistical analysis was corrected for the difference in life weight. This distinction in electrical resistance has been observed before and the higher content of highly resistant abdominal fat in females has been held responsible (Rawles et al., 1995). Alternatively, the smaller diameter of the females' legs may lead to a weaker contact between feet and shackle, thus increasing resistance. The lower effective current resulted in a significantly lower stunning efficiency of female broilers compared to males treated with the same voltage setting, across all stunning groups (Prinz et al., 2009d unpublished). If male and female broilers are being stunned in the same line, the voltage which is required for adequate stunning of females may cause meat quality problems in males due to the higher current. Constant current stunners could eliminate this concern, but they are currently not available for commercial use.

CONCLUSION

Application of external EEG electrodes with the CHEC has proved to be a useful method to record brain waves from a great number of broiler chickens following electrical waterbath stunning. Together with the construction of a typical base-line EEG this enables assessment of many different electrical stunning setups under the same experimental conditions.

The present study reveals some aspects of the complex interrelation of electrical waveform, amount and frequency of the current, application time and individual resistance of the broilers. All parameters significantly influence the induction of unconsciousness and the occurrence of physical reflexes. However, further investigations are necessary to understand the effect of the different electrical parameters on the neuronal and muscular tissue of the birds and thus optimise electrical stunning.

Although the interaction of the factors is now better understood, assessment of stunning efficiency with a specific combination of the stunning parameters is a prerequisite to ensure good welfare standards in commercial slaughterhouses. The following conclusions from the present experiment can be used as benchmarks.

Both, AC and pulsed DC stunning showed good stunning effectiveness. Stunning frequency has the biggest impact on the induction of unconsciousness. A maximum frequency of 400 Hz should not be exceeded for the highest tested stunning current of 150 mA for both waveforms, rectangular AC or pulsed DC. If a rectangular AC is applied, 400 Hz can also be applied in combination with 120 mA. The lowest acceptable stunning current for rectangular AC stunning is 100 mA, with a maximum frequency of 200 Hz. Currents below 100 mA can not be recommended for rectangular AC stunning. For pulsed DC stunning, currents ranging from 80 to 120 mA can be applied with a maximum frequency of 200 Hz. In combination with 150 mA pulsed DC, frequency can be increased to a maximum of 400 Hz. The minimum stunning time for the recommended pulsed DC setups is 10 seconds. The effect of stunning time on the induction of unconsciousness was not specifically investigated, but the effectiveness of pulsed DC stunning might be influenced by the application time of the current. Stunning time does not seem to have an influence on AC stunning. The waveform, sine or square wave, on the other hand significantly influences stunning efficiency of AC treatments. With lower current levels a sine wave AC is more effective compared to a rectangular AC.

Application of an AC has a higher impact on cardiac function, compared to pulsed DC. When AC of a low frequency was applied, good stunning efficiency coincided with cardiac arrest in the majority of animals. In the Simmons step-up system the AC in Phase II was less effective following a pulsed DC, while the majority of animals recovered from stunning. The Simmons step-up stunner did not show adequate stunning efficiency with 60V sine wave AC. It can however be assumed that a higher voltage setting in Phase II would improve stunning efficiency. This should be investigated in a separate study.

Absence of the corneal reflex indicates deep unconsciousness or approaching death, whereas a positive response is not necessarily related to consciousness and sensitivity. A prevalence of corneal reflexes in more than 30% of the animals or a sharp increase of positive responses indicates rapid recovery of the animals and inadequate stunning efficiency. The same applies if spontaneous eye blinking is visible in more than 15% of the birds within 20 seconds post-stun or 30% at 30 seconds post-stun. If a Simmons step-up stunner is used, eye reflexes cannot be used for assessment of the stunning effect, as physical reflexes are obviously suppressed with this stunning method. It is assumed that application of a low voltage, high frequency pulsed DC in Phase I is responsible for this effect, but the physiological reason remains unclear. This should be investigated in a separate study.

The occurrence of wing flapping and convulsions is considerably high in all approved stunning setups with a slightly lower level for pulsed DC stunning. A negative effect on meat quality cannot be excluded, but this should be assessed in a separate study. The Simmons step-up stunner does not cause convulsions or wing flapping in a great number of birds and might therefore be preferential for meat quality. It is thought that the pulsed DC in Phase I causes this distinction. It must be investigated if this positive effect can be maintained with higher voltages to ensure adequate stunning.

Female broilers showed a significantly higher electrical resistance, resulting in a significantly lower stunning current than males with the same constant voltage. Consequently, stunning efficiency of females was markedly reduced compared to males with the same voltage setting. This causes welfare concerns for constant voltage stunners. Constant current stunners could solve this problem, and their feasibility for commercial slaughterhouses should be further investigated. With constant voltage stunners, the voltage setting must be selected to ensure adequate unconsciousness in female broilers, probably causing excessive current levels in male birds. This might lead to meat quality problems.

Effective electrical waterbath stunning of broilers requires a good understanding of the influence of the electrical parameters and their interrelation in a setup. If a specific setup is selected and assessed with welfare criteria in mind, electrical waterbath stunning is a viable method to maintain welfare standards in commercial slaughterhouses.

Table 1: Recommendations for acceptable current-frequency combinations for electrical waterbath stunning in broiler slaughterhouses. The recommendations are subject to additional welfare assessment of the specific setup.

	70 Hz	100 Hz	200 Hz	400 Hz	800 Hz	1500 Hz
Rectangular AC [mA]	100-150	100-150	100-150	120-150	> 150	> 150
Pulsed DC [mA]	80-150	80-150	80-150	150 mA	> 150	> 150

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Summary

Electrical waterbath stunning of broiler chickens is the standard method to render birds unconscious before slaughter. A variety of electrical setups are used, differing in type and amount of current, voltage, frequency and stunning time. The influence of the electrical parameters on the induction of unconsciousness and their interrelation is not well understood. Moreover meat quality defects have been reported with high current levels. The aim of the present study was to analyse the effect of a range of electrical setups on the induction of unconsciousness in broiler chickens. For the assessment EEG analysis and physical reflexes were used. Recording of brain waves is the most objective available method to analyse the state of (un)consciousness.
In Chapter 1 a non-invasive EEG device, the chicken EEG clamp (CHEC), was developed. The CHEC allows fast and easy EEG recording from a great number of birds, facilitating systematic analysis of various electrical setups. In quantitative EEG analysis, the occurrence of an iso-electric EEG with less than 10% of the pre-stun brain power indicates good stunning efficiency. Two frequency bands have been considered: iso-electricity in the 2-30 Hz band is a sign of brain failure, whereas a profound suppression of the 13-30 Hz band indicates loss of sensibility. Using the CHEC, the spectral characteristics of wake broiler chickens were analysed in Chapter 2. Calculation of Fast Fourier Transformations (FFTs) revealed the typical base-line brain power of wake broilers. In the following stunning experiments, the post-stun brain power was expressed as a percentage of this representative base-line EEG.

For the stunning experiment 1321 male and female Ross broiler chickens were raised in one flock and allocated to 75 experimental groups. Single birds were stunned in an electrified waterbath. Subsequently the EEG was recorded for 120 seconds post-stun using the CHEC. Simultaneously the occurrence of spontaneous breathing, eye blinking and wing flapping was assessed and marked on separate observation channels on the EEG. The corneal reflex was tested every 20 seconds. Calculation of FFTs delivered the brain power in three post-stun periods: P1 0-20 seconds, P2 20-30 seconds and P3 30-40 seconds. These values were expressed as a percentage of the base-line EEG power. A level below 10% of the base-line brain power indicated induction of unconsciousness, while a higher percentage was sign of inadequate stunning. Failure of resumption of breathing was used as an indicator of cardiac arrest.

In the first stunning study in Chapter 3 the influence of different amounts and frequencies of a square wave alternating current (AC) was analysed with a stunning time of 10 seconds. A commercially available constant voltage stunning cabinet “Quest” was used to apply 60, 80, 100, 120 and 150 mA at frequencies of 70, 100, 200, 400 and 1500 Hz. AC showed good stunning results. Frequency had a major influence on the induction of unconsciousness. Setups with more than 400 Hz AC did not achieve good stunning effectiveness even with the highest tested current of 150 mA and can therefore not be recommended. A frequency of 400 Hz only showed adequate stunning effectiveness with a minimum current of 120 mA AC. Currents below 100 mA were not effective even with a low frequency of 70 Hz. Frequencies of 70, 100 and 200 Hz induced cardiac arrest in the majority of animals. At least 50% of the animals in groups that achieved good stunning results with AC expressed severe wing flapping following stunning. This might be an indicator for convulsions and negatively affect meat quality.

In the second stunning study in Chapter 4 the same electrical setups were analysed using a pulsed direct current (DC) with a mark:space ratio of 1:1. DC showed good stunning results. Similar to AC, frequency had a major influence on the induction of unconsciousness. Setups wit more than 400 Hz were not effective even with the highest tested current of 150 mA and can not be recommended. A frequency of 400 Hz can only be recommended in combination with 150 mA pulsed DC. A minimum current of 80 mA showed adequate stunning result with a maximum of 200 Hz. The occurrence of cardiac arrest was lower with DC stunning, 80% of the birds survived the stunning process with 80 mA at 200 Hz. Wing flapping as indicator for convulsions occurred in 40-60% of the animals in groups with adequate stunning results.
For both, AC and DC stunning, eye reflexes showed the best results to assess stunning efficiency. Some reflexes can still be visible in insensible birds. Positive corneal reflexes can be accepted in a maximum of 30% of the animals. Spontaneous eye blinking should not exceed 15% at 20 seconds post-stun and 30% at 30 seconds post stun.

In the third stunning study in Chapter 5 the influence of three different waveforms on male and female broiler chickens was analysed. Ten animals of each gender were stunned with either sine wave AC of 50 Hz, rectangular AC of 70 Hz or pulsed DC of 70 Hz with constant voltage levels of 60, 80 and 120 V. The same experimental setup was used as in the previous experiments, but stunning time was reduced to 4 seconds. In a constant voltage stunner, the actual current per broiler depends on the individual electrical resistance. Due to a significantly higher resistance, females obtained lower currents than males with the same constant voltage, resulting in a lower stunning efficiency. Constant current stunners should therefore be

considered for animal welfare reasons. The waveforms also showed a significant influence on the induction of unconsciousness. Sine wave AC was most effective with a minimum current of 70 mA. For rectangular AC a minimum of 90 mA could be established, while the pulsed DC treatments only showed acceptable results with 130 mA. The low stunning effectiveness of DC is in contrast to the results of the previous experiment. The reason for the difference may be the shorter stunning time. This indicates the importance of the application time of a pulsed DC for stunning efficiency. A stunning time longer than four seconds is therefore recommended for commercial slaughterhouses.

In the fourth stunning experiment in Chapter 6 the effectiveness of the Simmons step-up stunner was analysed. In Phase I a 550 Hz pulsed DC was applied in a shallow waterbath for 10 seconds, immediately followed by Phase II delivering a 50 Hz sine wave AC for 5 seconds on a metal plate. In Phase I a voltage of 12 and 15 V were applied followed by 40, 50 or 60 V sine wave in Phase II. Each stunning group consisted of 10 male and 10 female chickens. Assessment of stunning efficiency was performed as described for the previous experiments. Increasing stunning current in Phase II showed the biggest influence on improved stunning efficiency. The maximum level of 60 V however did not achieve adequate stunning results and higher voltages must therefore be applied. The majority of broilers recovered from stunning in all treatments. Although the EEG analysis indicated sensibility, eye reflexes were suppressed and can therefore not be recommended as indicators for the assessment of stunning effectiveness with the Simmons step-up stunner. Both effects are in contrast to findings with single phase sine wave AC of 60 V and the application of a high frequency pulsed DC in Phase I of the step-up stunner is thought to be responsible. The occurrence of wing flapping and convulsions was very low in the treatments with the step-up stunner, which might be an indicator for improved meat quality. It should be verified if this positive effect can be maintained with a higher voltage setting, which is necessary to ensure good animal welfare standards.

Both, AC and DC show a similar effectiveness regarding the induction of unconsciousness. The maximum acceptable frequency with 150 mA is 400 Hz for both waveforms. Adequate stunning time is an important factor for effective DC stunning, while it does not influence the effectiveness of AC. The Simmons step-up stunner did not show adequate results with a maximum of 60 V in Phase II. The higher electrical resistance of female broilers leads to a lower stunning efficiency with the same constant voltage treatment compared to males.

Constant current stunners could solve this problem. Electrical waterbath stunning of broiler chickens can be a viable method to maintain welfare standards in commercial slaughterhouses, if the electrical setups are selected and operated with welfare criteria in mind.

Samenvatting

Elektrische verdoving met gebruik van een onder stroom staand waterbad is in het slachthuis de meest toegepaste methode om pluimvee te bedwelmen. Elektrische grootheden als het type stroom, de hoeveelheid, de spanning, de frequentie en de verdovingstijd zijn daarbij van belang, maar worden naar believen gevarieerd. Dit leidt tot een groot aantal variaties in gebruikte stroomconfiguraties. De effecten van al deze parameters en hun wisselwerking op de verdoving zijn echter niet goed bekend. Er kan onvoldoende verdoving optreden, terwijl een te sterke stroom een negatief effect op de vleeskwaliteit kan hebben. Het doel van het hier gepresenteerde onderzoek is om de invloed van diverse elektrische grootheden op de effectiviteit van de bedwelming na te gaan. De meting van de elektrische activiteit in de vorm van een electroencephalogram (EEG), is de meest objectieve methode om de diepte van een verdoving te bepalen en staat dan ook centraal in dit proefschrift. Vele analyses van het EEG van bedwelmde dieren zijn verricht. Eveneens zijn meer traditionele maten om een verdoving te bepalen zoals een aantal fysieke reflexen, geregistreerd.

In Hoofdstuk 1 is een instrument ontwikkeld waarmee het EEG op een niet- invasieve manier gemeten kan worden. Met de ´chicken EEG clamp´ (CHEC) kan het EEG van een dier als een kip, op een eenvoudige, maar snelle, manier gemeten worden. Dit maakt het mogelijk om op een systematische wijze de effecten van diverse elektrische parameters bij de bedwelming van kippen te onderzoeken. Bij verdoving speelt iso- elektriciteit van het EEG een cruciale rol. Bij een iso- elektrisch EEG is geen activiteit meer zichtbaar en algemeen wordt aangenomen dat zo'n EEG een diepe verdoving met bewusteloosheid aangeeft. Bij een kwantitatieve EEG analyse wordt het EEG iso- elektrisch genoemd, indien het minder dan 10% van de oorspronkelijke basale EEG- activiteit omvat. Bij het EEG worden vaak twee frequentie-banden onderscheiden: een bredere band van 2-30 Hz, die de algemene graad van bewustzijn van het subject aangeeft, en een smallere band van 13-30 Hz, die pijngevoeligheid en sensibiliteit aanduidt. Iso- ektriciteit in de band van 2-30 Hz is een teken van een falende hersenactiviteit en naderende hersendood, terwijl iso- elektriciteit in de smallere band van 13-30 Hz op het ontbreken van sensorische gevoeligheid wijst.

In Hoofdstuk 2 zijn de typerende spectrale EEG karakteristieken van wakkere (niet-verdoofde) slachtkuikens met de CHEC onderzocht. Met een Fast Fourier Transformatie (FFT) is de basale EEG spanning van deze dieren vastgesteld. In al het volgende onderzoek is de gemiddelde basale spanning van wakkere dieren gebruikt als een referentiepunt, waartegen de reductie van het EEG als gevolg van de verdoving is berekend. Bij het verdovingsonderzoek zijn 1321 mannelijke en vrouwelijke slachtkuikens gebruikt en at random over 75 groepen verdeeld. Alle dieren zijn in het waterbad elektrisch bedwelmd.

Direct na verdoving is het EEG van alle dieren met de CHEC gemeten en wel gedurende 120 seconden. Tegelijkertijd is het optreden van ademhaling, van spontane oogbewegingen en van vleugelslagen gemarkeerd en geregistreerd. Ook is de sterkte van de cornea- reflex elke 20 seconden bepaald. Door een FFT is de power van elk EEG na verdoving berekend in tijdsperioden van 0 tot 20 seconden, van 20 tot 30 seconden en van 30 tot 40 seconden. De EEG spanning is steeds als percentage van de basale hersenspanning, die uit Hoofdstuk 2 bekend is, uitgedrukt. Waarden onder de 10% zijn een teken van een adequate verdoving en bewusteloosheid, terwijl hogere waarden wijzen op een niet voldoend diepe verdoving. Het niet meer ademhalen van dieren is als een indicator voor hartstilstand beschouwd.

In het eerste verdovingsonderzoek, dat beschreven is in Hoofdstuk 3, is de werking van verschillende sterkten en frequenties van wisselstroom onderzocht. Een verdovingstijd van 10 seconden is hierbij gebruikt. Een in de handel verkrijgbare elektrische instelkast ‘Quest’, die constante spanningen levert, is gebruikt om de kippen met 60, 80, 100, 120 en 150 mA bij frequenties van 70, 100, 200, 400, 800 en 1500 Hz te verdoven. Met wisselstroom zijn goede bedwelmingsresultaten verkregen, waarbij de frequentie van de stroom de grootste invloed op de verdovingsdiepte heeft. Bij verdovingen waarbij meer dan 400 Hz gebruikt is, worden geen goede resultaten meer bereikt, zelfs niet met een maximale stroomsterkte van 150 mA. Stromen met een dergelijk hoge frequentie kunnen derhalve niet aanbevolen worden. Zelfs met een frequentie van 400 Hz moet een minimale stroomsterkte van 120 mA toegediend worden om verzekerd te zijn van een voldoende verdoving. Met stroomsterkten onder 100 mA is met een lage frequentie van 70 Hz ook geen voldoende bedwelming meer te induceren. Frequenties van 70 tot 200 Hz, die de voorkeur verdienen, veroorzaken bij de meeste dieren een hartstilstand. Minstens 50% van de slachtkuikens in de groepen met een goede verdoving laten hevige vleugelslagen zien als ze uit het waterbad komen. Dit zou een teken van krampaanvallen kunnen zijn en dit zou de vleeskwaliteit kunnen aantasten.

In het tweede verdovingsexperiment, beschreven in Hoofdstuk 4, zijn pulserende gelijkstromen gebruikt en zijn dezelfde elektrische grootheden, als sterkte, spanning, frequentie en verdovingstijd, onderzocht. Met een dergelijk pulserende gelijkstroom zijn goede resultaten verkregen en net zoals bij wisselstroom heeft de frequentie het grootste effect op de bedwelming. Verdovingen met meer dan 400 Hz geven geen goede resultaten meer, zelfs bij een maximaal gebruikte stroomsterkte van 150 mA. Dergelijk hoge frequenties kunnen dus niet gebruikt worden.

Bij een frequentie van 400 Hz moet minimaal 150 mA pulserende gelijkstroom ingezet worden, terwijl bij een frequentie van 200 Hz een minimale stroomsterkte van 80 mA een voldoend diepe verdoving laat zien. Bij verdovingen met pulserende gelijkstroom komen hartstilstanden veel minder voor. Met zo'n stroom overleven 80% van de kippen een verdoving met 80 mA en 200 Hz. Wel treden hevige vleugelslagen op als teken van convulsies en krampen bij 40 - 60% van de goed verdoofde dieren.

Voor beide stroomtypen, wisselstroom en gelijkstroom, vormen oogreflexen een betrouwbare graadmeter bij de beoordeling van de verdoving. De afwezigheid ervan duidt op een voldoende verdoving. Niettemin kunnen deze reflexen soms bij goed verdoofde dieren ook optreden. Zo worden bij maximaal 30% van de verdoofde dieren cornea- reflexen geaccepteerd. Tot 20 seconden na verdoving kunnen echter bij niet meer dan 15% van de kippen spontane oogbewegingen geaccepteerd worden, terwijl tot 30 seconden na verdoving dat maar bij maximaal 30% van de dieren het geval mag zijn.

In het derde verdovingsonderzoek, beschreven in Hoofdstuk 5, is de invloed van diverse stroomtypen op mannelijke en vrouwelijke slachtkuikens geanalyseerd. In iedere groep zijn 10 mannelijke en 10 vrouwelijke dieren, ofwel met een sinusvormige wisselstroom van 50 Hz, ofwel met een rechthoekig pulserende wisselstroom van 70 Hz, ofwel met een pulserende gelijkstroom van 70 Hz, elk met constante spanningen van 60, 80 of 120 V, bedwelmd. Hetzelfde onderzoeksdesign als in de voorgaande studies is gebruikt, alleen werd een kortere verdovingstijd van vier seconden. Bij een constante spanning is per dier de stroomsterkte uit de individuele elektrische weerstand verkregen. Vrouwelijke dieren blijken een significant hogere weerstand dan mannelijke dieren te hebben. Dat is de reden dat hennen in alle onderzoeksgroepen bij eenzelfde spanning een lagere stroomsterkte dan hanen krijgen. Bij gelijke spanning resulteert dit een mindere verdoving van de hennen. Uit het oogpunt van dierenwelzijn zou verdoving met een constante stroomsterkte verricht dienen te worden; dit om eenzelfde verdoving bij mannelijke en vrouwelijke dieren te waarborgen. Vervolgens laten de diverse stroomsoorten ook een significant effect op de verdovingsdiepte zien. Een sinusvormige wisselstroom geeft de beste resultaten bij een verdoving met 70 mA en 50 Hz, terwijl een pulserende wisselstroom goede resultaten geeft bij een minimale stroomsterkte van 90 mA bij 70 Hz. Een pulserende gelijkstroom levert de beste resultaten bij een stroomsterkte van 130 mA bij 70 Hz. De minder goede verdoving die een pulserende gelijkstroom bij lagere stroomsterkten geeft, staat in contrast met eerder verkregen resultaten. Het verschil is naar alle waarschijnlijkheid terug te voeren op de kortere verdovingstijd van vier seconden. Dit laat ook de invloed van de verdovingstijd op de effectiviteit van een pulserende gelijkstroom zien. Daarom wordt ook een langere verdovingstijd dan vier seconden voor de praktijk aanbevolen.

In Hoofdstuk 6 is de verdoving die verkregen wordt met de twee- fasen ‘Simmons Stunner‘ onderzocht. Dit apparaat bestaat uit twee delen: een waterbad met een laagje water en een metalen plaat. Een pulserende gelijkstroom met een hoge frequentie van 550 Hz wordt over het waterbad gezet en een sinusvormige wisselstroom van 50 Hz over de metalen plaat. In Fase I wordt het dier met zijn kop door het waterbad gesleept, waarbij lage spanningen van 12 tot 15 V gebruikt worden, terwijl in Fase II de kop van het dier over de metalen plaat die onder spanning van 40, 50 of 60 V staat, gesleept wordt. In iedere groep zijn 10 mannelijke en 10 vrouwelijke dieren onderzocht. De beoordeling van de effectiviteit van de bedwelming is analoog aan de hiervoor beschreven onderzoekingen. Hogere spanningen in Fase II blijken het grootste effect op de verdoving te hebben, maar zelfs de hoogst onderzochte spanning van 60 V blijkt nog niet voldoende te zijn voor een acceptabel verdovingsresultaat. Daartoe zullen hogere spanningen gebruikt moeten worden. De meeste kippen komen na verdoving met de Simmons Stunner weer snel bij. In overeenstemming hiermee geeft het EEG sensibiliteit aan, maar zijn de oogreflexen onderdrukt. Deze kunnen bij deze methode dan ook niet gebruikt worden voor de beoordeling van de verdovingsdiepte. De effecten zijn niet geheel in overeenstemming met de resultaten uit de een- fase verdoving met 60 V sinusvormige wisselstroom. Het vermoeden is dat het gebruik van de hoogfrequente pulserende gelijkstroom in Fase I verantwoordelijk zou kunnen zijn voor de verschillen. Daarentegen is bij alle dieren die verdoofd zijn met de Simmons Stunner het optreden van vleugelslagen en krampen sterk gereduceerd. Dit is waarschijnlijk positief voor de vleeskwaliteit. Het verdient dan ook aanbeveling om na te gaan of dit positieve effect eveneens met hogere spanningen aanwezig blijft; hogere spanningen die voor de aanvaardbaarheid van deze verdovingsmethode nodig zijn.

Beide stroomtypen, wisselstroom en gelijkstroom, hebben een vergelijkbare effectiviteit op de bedwelming. Bij een stroomsterkte van 150 mA is een maximale frequentie van 400 Hz voor beide stroomsoorten toelaatbaar. Een niet te korte verdovingstijd is belangrijk om een adequate verdoving met gelijkstroom te krijgen, terwijl deze tijd bij wisselstroom vrijwel geen invloed heeft. Met de twee- fasen Simmons Stunner wordt geen voldoende verdoving verkregen met een spanning van 60 V wisselstroom in Fase II. De hogere elektrische weerstand van vrouwelijke dieren leidt tot een minder diepe verdoving in vergelijking met mannelijke slachtkuikens. Een verdovingsinstallatie die met constante stroomsterktes werkt, zou dit probleem kunnen verhelpen. Al met al moet de conclusie zijn dat bij een zorgvuldige keuze van elektrische grootheden de verdoving van pluimvee in slachthuizen met het waterbad, gezien in het licht van dierenwelzijn, acceptabel en toelaatbaar is.

Zusammenfassung

Die elektrische Betäubung im Wasserbad ist die in der Praxis am häufigsten verwendete Methode um Geflügel vor der Schlachtung zu betäuben. Die elektrischen Parameter wie Stromart und -menge, Spannung, Frequenz und Betäubungszeit werden beliebig variiert. Dies führt zu einer großen Vielfalt von Kombinationen. Der Einfluss der einzelnen Parameter und ihre Wechselwirkung sind dabei nicht ausreichend bekannt. Hohe Ströme können außerdem einen negativen Effekt auf die Fleischqualität haben. Das Ziel der vorliegenden Studie ist es, den Einfluss vieler verschiedener elektrische Parameter auf die Betäubungseffektivität zu untersuchen. Zur Beurteilung wurden Hirnstrom-Analysen und physische Reflexe herangezogen. Die Messung von Hirnströmen ist die objektivste verfügbare Methode zur Beurteilung der Betäubungstiefe. Im ersten Kapitel wurde ein Gerät zur nicht-invasiven Messung von Elektroencephalogrammen (EEGs) hergestellt, die Hähnchen EEG-Zange (CHEC). Mit der Zange können EEGs von Masthähnchen schnell und einfach aufgezeichnet werden. Dies erleichtert die systematische Untersuchung vieler Betäubungsparameter. In der quantitativen EEG-Analyse wird das Auftreten eines iso-elektrischen EEGs mit weniger als 10% der ursprünglichen Hirnspannung als Zeichen für tiefe Bewusstlosigkeit bewertet. Dabei werden zwei Hirn-Frequenzbereiche betrachtet: Iso-Elektrizität im 2-30 Hz Bereich ist ein Zeichen für fehlende Hirnaktivität oder den nahen Hirntod. Eine deutliche Reduktion auf weniger als 10% der Basis-Hirnspannung im 13-30 Hz Bereich signalisiert fehlende Sensibilität. Im zweiten Kapitel wurden die typischen spektralen Merkmale von wachen Hähnchen mit der Hähnchen EEG-Zange untersucht. Mittels Fast Fourier Transformationen (FFTS) wurde die typische Basis-Hirnspannung wacher Masthähnchen ermittelt. In den folgenden Betäubungsversuchen wurde diese Basis-Hirnspannung als Referenz verwendet, um die prozentuale Reduktion der Hirnspannung nach der Wasserbad Betäubung zu berechnen.

Für die Betäubungsversuche wurden 1321 männliche und weibliche Masthühner in einer Herde aufgezogen, und anschließend zufällig in 75 Betäubungsgruppen verteilt. Die Tiere wurden einzeln im Wasserbad betäubt. Anschließend wurden die EEGs mit der Hähnchen EEG-Zange für eine Dauer von 120 Sekunden aufgezeichnet. Gleichzeitig wurde das Auftreten von Atmung, spontanem Augenzwinkern und Flügelschlagen auf separaten Observationskanälen auf dem EEG markiert. Der Kornealreflex wurde alle 20 Sekunden getestet. Mittels FFTs wurde die Hirnspannung in drei verschiedene Zeiträume nach der Betäubung berechnet: P1 (0-20 Sekunden), P2 (20-30 Sekunden) und P3 (30-40 Sekunden). Diese Hirnspannung wurde als Prozentsatz der Basis-Hirnspannung ausgedrückt. Werte unter

10% wurden als Zeichen für tiefe Bewusstlosigkeit bewertet, während ein höherer Wert ungenügende Betäubung anzeigte. Das Ausbleiben der Atmung nach der Betäubung war ein Indikator für Herzstillstand.

Im ersten Betäubungsversuch in Kapitel 3 wurde die Wirkung verschiedener Stromstärken und Frequenzen von Wechselstrom (AC) bei einer Betäubungszeit von 10 Sekunden analysiert. Ein handelsüblicher elektrischer Steuerkasten „Quest" für konstante Spannungen wurde verwendet, um die Broiler mit 60, 80, 100, 120 und 150 mA bei Frequenzen von 70, 100, 200, 400, 800 oder 1500 Hz zu betäuben. Mit Wechselstrom konnten gute Betäubungsergebnisse erzielt werden. Die Frequenz hatte den größten Einfluss auf die Betäubungstiefe. Behandlungen mit mehr als 400 Hz erzielten keine guten Ergebnisse bis zur maximal verwendeten Stromstärke von 150 mA und können daher nicht empfohlen werden. Bei einer Frequenz von 400 Hz muss eine Mindest-Stromstärke von 120 mA appliziert werden, um eine ausreichende Betäubungstiefe zu gewährleisten. Stromstärken unter 100 mA zeigten auch mit niedrigen Frequenzen von 70 Hz keine ausreichende Wirkung. Frequenzen von 70 bis 200 Hz führten bei der Mehrheit der Tiere zum Herzstillstand. Mindestens 50% der Tiere in Gruppen mit guter Betäubungswirkung zeigten heftiges Flügelschlagen nach Verlassen des Wasserbades. Dies könnte ein Zeichen für Krämpfe sein und die Fleischqualität beeinträchtigen.

Im zweiten Versuch in Kapitel 4 wurden die gleichen elektrischen Parameter mit einem gepulsten Gleichstrom (DC) untersucht (Puls-Länge 1:1). Mit gepulstem DC konnten gute Betäubungsergebnisse erzielt werden. Wie beim Wechselstrom hatte die Frequenz den größten Einfluss auf die Betäubungstiefe. Behandlungen mit mehr als 400 Hz erzielten keine guten Ergebnisse bis zur maximal verwendeten Stromstärke von 150 mA und können daher nicht empfohlen werden. Bei einer Frequenz von 400 Hz müssen mindestens 150 mA gepulster DC eingesetzt werden. Eine Mindest-Stromstärke von 80 mA zeigte gute Ergebnisse bis zu einer Frequenz von 200 mA. Das Auftreten von Herzstillstand war geringer bei den Behandlungen mit gepulstem Gleichstrom. 80% der Tiere überlebten die Betäubung bei 80 mA und 200 Hz gepulstem DC. Heftiges Flügelschlagen als Zeichen von Krämpfen trat bei 40-60% der Tiere in Gruppen mit guter Betäubungswirkung auf.

Für beide Stromarten, Wechselstrom und Gleichstrom, zeigten Augenreflexe die zuverlässigsten Ergebnisse zur Beurteilung der Betäubungstiefe. Einige Reflexe können auch bei gut betäubten Tieren auftreten. Bei maximal 30% der Tiere können daher Kornealreflexe akzeptiert werden. Nicht mehr als 15% der Tiere sollten spontanes Augenzwinkern 20 Sekunden nach Betäubungsende zeigen, 30 Sekunden nach der Betäubung sollte dies bei maximal 30% der Broiler auftreten.

Im dritten Betäubungsversuch in Kapitel 5 wurde der Einfluss verschiedener Stromarten auf männliche und weibliche Broiler analysiert. Je 10 männliche und weibliche Tiere wurden mit sinusförmigem Wechselstrom von 50 Hz, rechteckförmigem Wechselstrom von 70 Hz oder gepulstem Gleichstrom von 70 Hz mit konstanten Spannungen von 60, 80 oder 120 V betäubt. Es wurde der gleiche Versuchsaufbau verwendet wie in den vorherigen Studien, mit einer kürzeren Betäubungszeit von vier Sekunden. Bei konstanter Betäubungsspannung ergibt sich die Stromstärke pro Tier aus dem individuellen elektrischen Widerstand der Broiler. Durch einen signifikant höheren elektrischen Widerstand erhielten die weiblichen Tiere in allen Versuchsgruppen eine niedrigere Stromstärke als die mit gleicher Spannung behandelten männlichen Broiler. Daraus resultierte eine niedrigere Betäubungstiefe der weiblichen Hühner. Aus Tierschutzgründen sollten daher Betäubungsanlagen mit konstanter Stromstärke pro Tier eingesetzt werden. Die verschiedenen Stromarten zeigten ebenfalls einen signifikanten Einfluss auf die Betäubungstiefe. Sinusförmiger AC erzielte die besten Ergebnisse mit einer guten Betäubungseffektivität bei 70 mA bei 50 Hz. Für rechteckigen AC muss eine Mindest-Stromstärke von 90 mA bei 70 Hz verwendet werden. Gepulster Gleichstrom erzielte gute Betäubungsergebnisse erst bei einer Stromstärke von 130 mA bei 70 Hz. Die schlechte Betäubungswirkung von gepulstem DC steht im Gegensatz zu den zuvor erläuterten Ergebnissen. Der Unterschied könnte auf die kürzere Betäubungszeit von vier Sekunden zurückzuführen sein. Dies zeigt die Bedeutung der Durchströmungszeit für die Betäubungseffektivität von gepulstem Gleichstrom. Eine Betäubungszeit von mehr als vier Sekunden wird daher für die Praxis empfohlen.

Im sechsten Kapitel wurde die Betäubungswirkung des zweiphasigen Simmons Betäubers untersucht. In Phase I wird ein gepulster Gleichstrom von 550 Hz in einem flachen Wasserbad angelegt, gefolgt von einem sinusförmigen Wechselstrom von 50 Hz in Phase II, der über eine Metallplatte appliziert wird. In Phase I wurden Spannungen von 12 und 15 V verwendet, gefolgt von 40, 50 oder 60 V in Phase II. In jeder Gruppe wurden je 10 männliche und 10 weibliche Tiere betäubt. Die Beurteilung der Betäubungseffektivität erfolgte analog zu den zuvor beschriebenen Versuchen. Höhere Spannungen in Phase II hatte den größten Einfluss auf eine verbesserte Betäubungswirkung. Die höchste untersuchte Spannung von 60 V war allerdings nicht ausreichend um gute Betäubungsergebnisse zu erzielen. Höhere Spannungen müssen daher verwendet werden. Die Mehrzahl der Broiler erholte sich schnell nach der Betäubung in allen Versuchsgruppen. Während die EEG-Analyse Sensitivität anzeigte, waren die Augenreflexe unterdrückt und können daher nicht zur Beurteilung der Betäubungstiefe nach Betäubung mit dem Simmons Betäuber heran gezogen werden. Beide Effekte stehen im Gegensatz zu den Ergebnissen der einphasigen Betäubung mit 60 V sinusförmigem AC. Es wird vermutet dass der Einsatz des hochfrequenten Gleichstroms in Phase I für den Unterschied verantwortlich ist. In allen Versuchsgruppen mit dem Simmons Stunner war das Auftreten von Flügelschlagen und Krämpfen sehr stark reduziert, was sich positiv auf die Fleischqualität auswirken kann. Es sollte daher untersucht werden, ob sich dieser positive Effekt auch mit höheren Spannungen bestätigt, da diese aus Tierschutzgründen nötig sind.

Beide Stromarten, Wechselstrom und gepulster Gleichstrom erzielten eine vergleichbare Betäubungswirkung. Bei einer Stromstärke von 150 mA ist eine maximale Frequenz von 400 Hz für beide Stromarten zulässig. Eine ausreichende Durchströmungszeit ist wichtig für eine gute Betäubungswirkung von gepulstem Gleichstrom, während sie keinen Einfluss auf Wechselstrombetäubung hat. Der zweiphasige Simmons Betäuber zeigte keine ausreichenden Ergebnisse mit einer Spannung von 60 V sinusförmigem AC in Phase II. Der höhere elektrische Widerstand weiblicher Tiere führt zu einer schlechteren Betäubungswirkung im Vergleich zu männlichen Broilern bei gleicher Spannung. Eine Betäubungsanlage mit konstanter Stromstärke könnte dieses Problem lösen. Bei sorgfältiger Auswahl und Kontrolle der elektrischen Parameter kann die Betäubung im elektrischen Wasserbad eine tierschutzgerechte Betäubung in Geflügelschlachthöfen gewährleisten.

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Curriculum Vitae

Simone Prinz was born in Oberhausen, Germany on 1st of August in 1978. She completed her secondary education (Gymnasium) in 1998. Following a one-year internship on a farm, she started her studies in agricultural sciences at the University of Hohenheim in Stuttgart, Germany. She obtained the degree of Bachelor of Science with specialisation in animal science from the University of Hohenheim, Department of Farm Animal Ethology and Poultry Production, in August 2002. She continued her studies at the University of Reading in England and graduated from there as Master of Science in Agricultural Management in September 2003. Thereafter she was responsible for quality assurance of poultry meat at Esca Food Solutions, an international supplier of meat products. She was involved in the assessment of animal welfare during broiler rearing and processing, which formed the basis for her PhD thesis. Supported by Esca Food Solutions she initiated a project with the University of Hohenheim in cooperation with the Radboud University Nijmegen in the Netherlands, to study the effectiveness of electrical waterbath stunning. This PhD thesis is the result of the research conducted in the project. With her expertise in poultry stunning she now works for the research department of Stork PMT in Boxmeer.

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